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# PSEUDOALLELISM AND THE GENE CONCEPT*

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It is characteristic of every field of investigation that its fundamental ideas and concepts are submitted continually to re-examination, and that when the available information warrants, these ideas may be either modified or rejected outright. Such is the case in genetics where the concept of the unit gene is perforce the subject of ceaseless scrutiny.

The discovery and investigation of instances of pseudoallelism occurring in Drosophila, Aspergillus and Neurospora, and in all probability in maize and cotton, have suggested that the commonly accepted ideas of the gene require modification. At the outset, then, an attempt will be made briefly to indicate the conventional implications of the gene concept and the demands for revision of the gene concept which have stemmed from the analysis of cases of pseudoallelism.

An overwhelming body of experimental and observational information has supported the notion that the chromosomal material can be delineated into particulate, self-perpetuating parts each of which may be designated as a gene. While the precise spatial limits of any particular gene remain unknown, it has been convenient to adopt crossing-over frequency as an empirical measure of the distance between contiguous genes. This relationship has been adopted because of the repeatability of cross-over frequencies and because the cross-over event appears to occur without altering the integrity of the gene.

The concept of the spatial gene must be supplemented with the idea that each gene may undergo mutation at a particular rate to one or more allelic forms without alteration in position and without change in the recombination relationships with its neighbors. The detection of this mutational event is based upon the fact that coincident with mutation to an allelic state a change in the functional role of the gene occurs which is ultimately manifest in the phenotype of the organism being studied.

Detailed studies of the specific biochemical and physiological alterations which accompany mutation have led to the generalization that in

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diploid organisms each gene has a specific functional role to play, one which is not duplicated by any other non-allelic gene.

While there are many gaps in our knowledge concerning the gene—the precise determination of gene size and the association of the gene with a known chemical entity; the exact mechanisms which trigger mutations and the specific chemical and/or physical changes which accompany mutation; or the details of the mechanisms wherein the genes exert their regulatory influence during the development of each organism—nonetheless, the bulk of data available prior to the discovery of pseudoallelism supported the hypothesis that the spatial gene, the mutational gene and the functional gene are one and the same. This may be designated as the unified gene concept.

It is appropriate to ask now: wherein has pseudoallelism necessitated a reconsideration of the unified gene concept? The instances of pseudoallelism described, regardless of the organism, fulfill the phenotypic criteria of allelism but fail to fulfill the genotypic criteria. Thus independent mutants exhibit similar or identical phenotypes, and heterozygotes compounded with the mutants manifest a phenotype either intermediate or inseparable from that of one or the other mutant. Yet recombination occurs between the mutants such that infrequently either both or neither segregate to the same gamete. The phenotypic data suggests that the mutants are functionally alike; the recombination data suggests that they are spatially contiguous.

Two further observations have motivated the suggestion that the unit gene concept as discussed requires modification. First, a striking difference has been observed when comparing the phenotypic response concomitant to the coupling ("cis") versus repulsion ("trans") genotypes. Where the pseudoalleles are recessive, the coupling genotype results in a wild-type phenotype, whereas the repulsion genotype results in a mutant phenotype. Second, in a few cases, e.g., the lozenge (Green and Green, 1949) and bithorax mutants (Lewis, 1951) in *D. melanogaster* and the biotinless mutants (Roper, 1950) in Aspergillus the recombination data demonstrate that more than two loci are involved. It is reasoned that explanations for these phenomena follow if a distinction is made between the limits of the spatial gene and the functional gene (Goldschmidt, 1950, 1951; Pontecorvo, 1952, 1953). Accordingly, pseudoallelic loci (i.e., spatial loci) represent components of a larger "physiological" gene, whose functions are indistinguishable but whose integral mutational sites are separable by crossing-over. The phenotypic differences associated with the coupling versus repulsion genotypes are readily explained in terms of the usual rules of dominance.

## THE FUNCTIONAL DISPARITY OF PSEUDOALLELIC MUTANTS

This dichotomy—the distinction between spatial and functional genes—poses certain questions. One obvious question which can be raised is: Does functional diversity occur among the mutants of a pseudoallelic

system? (Let it be made clear at this point that function and phenotype are not synonyms. There are numerous examples which demonstrate that similar or identical phenotypes can be arrived at by alteration of different steps in the developmental process, each such alteration being the result of a different, functional gene. Thus diversity of function may lead to identity of phenotype.) In certain cases the functional separability of pseudoallelic mutants is obvious. The Star-asteroid pseudoalleles in *D. melanogaster* are a case in point (Lewis, 1945). While these mutants fulfill the requisites of pseudoallelism, they are nonetheless functionally distinctive. Thus, Star mutants are dominant and homozygous lethal, while asteroid mutants are recessive and homozygous viable. These striking phenotypic differences can best be accounted for by the assumption that Star and asteroid mutants differ functionally despite the fact that the precise manner by which they differ has not been ascertained thus far. An identical interpretation applies when considering the Stubble-stubbloid pseudoalleles in *D. melanogaster* (Lewis, 1951). The former is a dominant, homozygous lethal; the latter is recessive and homozygous viable. In the case of the singed mutants of *D. melanogaster,* two types occur, female fertile and female sterile, thereby indicating functional distinctiveness (Bridges and Brehme, 1944; Ives and Noyes, 1951). The elegant analysis of the bithorax-bithoraxoid complex in *D. melanogaster* has demonstrated that while the phenotypes associated with these mutants bear a superficial similarity, they nevertheless manifest distinctive developmental effects on the halteres and thoracic segments of the fly (Lewis, 1951).

To summarize, the aforementioned cases of pseudoalleles manifest separable phenotypes which are best interpreted as functions of different developmental influences or alterations.

In certain other cases of pseudoallelism, the developmental distinctiveness is less obvious, but has been demonstrated by the use of so-called suppressor mutants. The well-known vermilion eye color mutants in Drosophila will serve as an example. A number of independent vermilion mutants behave alike in preventing the biosynthesis of brown eye pigment. All are developmentally non-autonomous, accumulate non-protein tryptophan, and synthesize eye pigment when supplied exogenously with either kynurenine or formylkynurenine (see Green, 1952, for literature summary). In short, all mutants appear incapable of utilizing tryptophan, the initial step in the biosynthesis of brown eye pigment. While the accumulated biochemical and developmental data point to the functional identity of the vermilion mutants, their behavior in the presence of an independent suppressor mutant attests to their functional discreteness. Thus some vermilion mutants are suppressible, i.e., their eye phenotype is modified in the direction of wild-type in the presence of the suppressor; while others are unsuppressible, i.e., their eye phenotype is not changed by the suppressor (Green, 1952, 1954). It is pertinent to add here that the suppressor mutant also functions to suppress certain other non-vermilion mutants, e.g., purple eye color (an autonomous eye color mutant), sable body color and speck body

color. Thus the suppressor mutant acts on certain non-vermilion mutants yet fails to influence some vermilion mutants. These facts lead to the inescapable conclusion that the suppressible and unsuppressible vermilion mutants are functionally distinctive despite the fact that they fulfill the phenotypic criteria of allelism. By applying the same suppressor technique, it has been demonstrated that the forked bristle mutants of *D. melanogaster* fall into two classes, suppressible and unsuppressible (Green, unpublished), and as will be discussed below are also pseudoallelic. Similar results are indicated for the inositolless and tryptophane-desmolase loci in Neurospora (Giles, 1951; Yanofsky, 1952).

In the case of mutants at the white locus, a somewhat different observation dictates the concept of functional discreteness among independent white mutants. It has been noted that the white alleles can be classified into two categories depending upon their interaction with the sex-linked, recessive eye color mutant zeste (Gans, 1953). Certain of the white alleles act as dominant suppressors of zeste, others do not. It follows from this classification that certain of the white pseudoalleles must differ functionally, i.e., the zeste suppressors differ from the non-suppressors.

These facts indicate that functional dissimilarity is the rule within a pseudoallelic complex. It is not difficult to understand how two independently occurring mutants each affecting the identical developmental step will lead to a mutant phenotype when both are included in the genotype. This is allelism. It is, however, not immediately obvious how this occurs when two independent mutants each affecting different, although admittedly intimately related, developmental steps do so. This is a basic issue manifest with the problem of pseudoallelism. It is not clear how the postulate of the "physiological" gene answers this question since such a postulate must also consider the functional disparity of pseudoalleles.

## THE CORRESPONDENCE BETWEEN THE FUNCTIONAL AND SPATIAL GENES

Since functional discreteness is indicated within pseudoallelic systems, it is of importance to know whether the functional units of each type are associated solely with one locus. The test of this association entails an analysis of the recombination relations of a number of independent mutants within any one pseudoallelic complex. If there is a necessary, inherent association between the functional gene and the spatial gene, then the recombination test would demonstrate that mutants associated with one function would recombine with mutants of the second function. The data available on this point are not voluminous, but they nonetheless support the notion that function and localization are not divisible by crossing-over. In the case of the Star-asteroid mutants, the recombination relations of three asteriod and two Star mutants have been determined; all the asteriod mutants recombine with one or the other Star mutant (Lewis, 1945). Thus it may be concluded that the asteriod mutants constitute one group of alleles and the Star mutants an independent group of alleles. Additional examples

which parallel and support the Star-asteriod case include the following. Among four singed mutants tested, the three female-sterile mutants recombine with the female-fertile mutant (Ives and Noyes, 1951; Hexter and Green, unpublished). Tests of six white mutants show that the five zeste-suppressing mutants recombine with a white mutant which fails to suppress zeste and thereby may be considered to be allelic (Mackendrick and Pontecorvo, 1952; Lewis, 1952). In the case of four forked mutants tested, the two suppressible mutants recombine with one or the other non-suppressible mutant (Green, unpublished).

Taken together these data support the concept of the functional and spatial identity of the gene, and demonstrate further that the integrity of this association is unaltered by the process of crossing-over.

It may be argued that while the criterion used to indicate non-allelism, i.e., crossing-over, is valid this does not necessarily establish the allelism of functionally inseparable independent mutants, and the only conclusive test is further testing for crossing-over between these latter mutants. Moreover, it can be reasoned that the crossing-over relations within a pseudoallelic complex are continuous rather than discontinuous, and allelism as such does not exist.

Logical difficulties are encountered when attempting to collect critical information on this point. Given two mutants both of which cross-over with a third, what number of individual gametes must be tested to be certain that the two mutants will not cross-over with one another? There is, of course, no way of determining this number nor of establishing confidence limits of the cross-over test. If, however, within a pseudoallelic complex a large number of independent mutants are subjected to the crossing-over test, and if they distribute themselves into finite loci, then it is reasonable to conclude that the results are valid. Such a test has been made in the case of the lozenge pseudoalleles in *D. melanogaster* where nineteen independent mutants have been tested (Green and Green, 1949, and unpublished). The results demonstrate that each mutant can be assigned, as a consequence of the crossing-over tests, to only one of the three lozenge loci, and thus indicate the discontinuous nature of the pseudoalleles.

## EVOLUTIONARY SEPARATION OF PSEUDOALLELES

Data have been collected which strongly suggest that mutants which occur as members of a pseudoallelic system in one species, *D. melanogaster,* occur as independent, unlinked mutants in a second species, *D. virilis.* As noted previously the vermilion mutants in *D. melanogaster* while phenotypically indistinguishable, can be classified into two types based upon their phenotypic behavior in the presence of a suppressor. Psuedoallelism is indicated since crossing-over has been obtained between suppressible and and unsuppressible mutants. A second method may be used to distinguish between vermilion mutants. It has been known that if vermilion larvae are "starved" at a critical period, brown eye pigment synthesis occurs (Tatum and Beadle, 1939). An investigation of this effect shows that independent

mutants differ in their response to starvation, and that the response parallels exactly the behavior with the suppressor. Thus larvae of suppressible vermilion mutants respond to starvation by forming brown eye pigment, but no response is elicited from unsuppressible vermilion larvae subjected to starvation (Shapard, 1954).

In *D. virilis* there occur two mutants which are biochemically inseparable from one another and from the vermilion mutants of *D. melanogaster.* These mutants, the sex-linked vermilion and the autosomal cardinal, both fail to synthesize brown eye pigment, accumulate non-protein tryptophan, are non-autonomous developmentally and synthesize brown eye pigment when exogenously supplied with kynurenine or formylkynurenine (Price, 1949; Green, 1952). In all respects they parallel exactly the vermilion pseudoalleles of *D. melanogaster.*

Is it possible to homologize the *melanogaster* and *virilis* mutants? An attempt has been made to establish this homology by determining the response of the *virilis* mutants to larval starvation and to the suppressor mutant. The larval starvation tests discriminate between the vermilion and cardinal mutants (Shapard, 1954). Thus following larval starvation, cardinal flies synthesize brown pigment while vermilion flies do not. Parallel results were obtained when the mutants were tested by the suppressor (H. W. Lewis and Green, unpublished). Since a suppressor mutant homologous to the suppressor of vermilion of *D. melanogaster* is not known for *D. virilis,* the *D. melanogaster* suppressor was used. This could be done by making use of the transplantation technique in which eye anlage from either vermilion or cardinal larvae were implanted into *D. melanogaster* larvae homozygous for both the suppressor and unsuppressible vermilion mutants. As a control implants were made into vermilion, non-suppressor hosts. Both hosts are incapable of synthesizing brown eye pigment. The results obtained parallel those in the "starvation" tests. Thus cardinal eye anlage implants react non-autonomously in the *melanogaster* host with the suppressor and synthesize a small, but discernible amount of brown pigment, but react autonomously in the non-suppressor host. On the other hand, the vermilion implants react autonomously in both *melanogaster* hosts. These results demonstrate that the cardinal mutant is suppressible while the vermilion mutant is not.

These observations considered collectively suggest that the cardinal mutant of *D. virilis* is the homologue of the suppressible vermilion mutants of *D. melanogaster* while the vermilion mutant of *D. virilis* is the homologue of the unsuppressible vermilion mutants of *D. melanogaster.* Or stated another way, mutants which exist as closely linked entities in one species occur as widely separate entities in a different species.

Alternatively, it may be suggested that the homology is superficial and that only coincidentally the *melanogaster* mutants behave like the *virilis* mutant. Consideration of the parallelisms and differences described herein suggests that this is asking too much of coincidence.

SUMMARY

The information assembled and submitted here supports the concept that the spatial gene, the functional gene and the mutational gene are one and the same. Moreover, pseudoallelism does not invalidate but rather augments the unified gene concept.

Thus while pseudoallelic mutants manifest similar or even inseparable phenotypes, the available evidence points to their functional distinctiveness. The occurrence in the case of pseudoalleles of a differential phenotype depending upon whether the mutants are associated in coupling or repulsion cannot be readily explained in terms of an all inclusive "physiological" gene within which crossing-over occurs. Rather, the explanation will follow when the mechanism of gene action, particularly of pseudoalleles, is better understood.

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# SOME ASPECTS OF POSITION PSEUDOALLELISM*

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## INTRODUCTION

The phenomenon of pseudoallelism promises to contribute much to our understanding of the gene—how it functions, how it mutates, how it evolves. The functional aspect will be the central theme of this paper. Attention will be focused chiefly on examples of "position pseudoallelism;" namely, those cases in which there is a position effect or phenotypic difference between the "cis-type" ($ab/++$) and "trans-type" ($a+/+b$) of double mutant heterozygote. In most of these examples a close functional relationship between the adjoining loci is indicated.

There are currently two contrasting interpretations of position pseudoallelism. On the first or functional interpretation the mutants at the different pseudoallelic loci are alterations at different sites of a single functional unit which is called the gene. On the second or genetic interpretation the mutants at the different loci are alterations in different units each of which is called a gene, whether it be a single functional unit or not.

The functional interpretation is currently advocated by Pontecorvo (1952) on the basis of studies (Roper, 1950; and Roper in Pontecorvo et al., 1953) of three biotin mutants in *Aspergillus nidulans*, which are presumptive position pseudoalleles although recovery of cis-types has not been reported; and by MacKendrick and Pontecorvo (1952) on the basis of studies of certain pairs of white "alleles," which, by analogy with the case of apricot and white (Lewis, 1952), may be assumed to be position pseudoalleles. The chief difficulty with this type of interpretation is that adequate criteria for recognizing a functional unit are not available. Mere appearance of functional identity, as in the case of the above biotin mutants, or certain inositolless mutants in Neurospora which are also presumptive position pseudoalleles (Giles, 1951), is obviously not sufficient. Thus, it is easy to see how two or more units which control different reactions in a sequential series can mimic the action of a single unit. Another criterion has been the phenotypic test of allelism; thus, two recessive mutants, each arising independently from a standard or wild type, have been considered alleles of a single gene if the heterozygote between them has a mutant phenotype. This criterion is inadequate since it does not take into account the possibility of position effects; thus, the finding that the trans-type in the case of position

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pseudoallelism is mutant in phenotype is readily explained by a position effect involving the products of two different functional units (Lewis, 1951).

Paradoxically, the application of the phenotypic test of allelism has largely been responsible for endowing the gene with complex functional attributes rather than a unity of function. Thus, most of the well-studied cases of multiple allelism (or pseudoallelism, as the case may be) that have been identified largely by means of this test show evidence for at least two, more or less independently varying, functional components; for example, the dumpy mutants (Muller, 1922) and the scute and achaete series of mutants (beginning with the work of Serebrovsky, 1930) in Drosophila, and the "*R*" mutants (Emerson, 1921; Stadler, 1946; 1953; 1954) and the "*A*" mutants (see Laughnan in this Symposium) in maize. The difficulties in setting up criteria for functional unity become more aggravated in the case of allelic or pseudoallelic series of dominant mutants and/or mutants of obscure origin. Such cases tend to show even greater degrees of complexity at the functional level; *e.g.*, self-sterility alleles in Oenothera (D. Lewis, 1949; and others), the "E" mutants in Bombyx (reviewed by Tanaka, 1953) and the genes controlling cellular antigens in cattle (Stormont, Owen and Irwin, 1951).

The evidence from established cases of position pseudoallelism in Drosophila suggests not a functional unity but as many functional components as there are different loci. This has been discussed in detail before (Lewis, 1951) for the cases of Star-asteroid (Lewis, 1945), Stubble-stubbloid, three loci of the bithorax series, and the three loci of the lozenge series investigated by Green and Green (1949). In the lozenge case the evidence is incomplete but already points to at least two functional components (fertility and eye effects). The more recent example of apricot-white pseudoallelism, referred to above, well illustrates the same point, since the series of white "alleles" has long been known (Morgan, et al., 1931) to be separable into two qualitatively distinct groups with respect to reaction to Bridges' specific modifier gene, Pale, or to sexual dimorphism. Thus, the eye-color of mutants of the "apricot" group (including apricot, blood, coral and honey) is darker in the male than the female, while it is lighter in the male than in the female in the case of the "eosin" group (including white, as well as eosin and most of the other mutants of the series). The data of MacKendrick and Pontecorvo (1952) can be interpreted to mean that coral and blood each lie to the left of white, parallelling the finding that apricot lies to the left of white. To this limited extent, at least, the separation of the white series by the crossing-over test coincides with the separation by the test of qualitatively different function. In the recent case of two vermilion position pseudoalleles, Green (1954) has used a more or less specific modifier gene to distinguish the two; but the evidence for functional differentiation is not quite so convincing in this case. Finally, there is the probable case of two singed position pseudoalleles discovered by Ives and Noyes (1951), which awaits report of the cis-type; here, too, the evidence from existing mutants

(see Bridges and Brehme, 1945) suggests two, more or less independently varying attributes (fertility and bristle effects).

By contrast the genetic interpretation refers the complexity of functioning which typifies pseudoallelism to interactions at the level of different gene products. The standard operational criteria for defining the gene—indivisibility by the crossing-over or rearrangement test—are thus still preserved; and there remains a one-to-one correspondence between gene and locus.

Specifically, a model of gene action in terms of sequential biochemical reactions has proved a fruitful working hypothesis. This kind of model was formerly advocated by Pontecorvo (1950) on the basis of theoretical considerations of millimicromolar reactions (McIlwain, 1946), and was independently put forward (Lewis, 1949) and elaborated in some detail (Lewis, 1950; 1951) to explain the position effect which characterizes position pseudoallelism. This model assumes that (1) the normal allele of one of the pseudoallelic genes, $a^+$, controls a reaction: S $\longrightarrow$ A, while the normal allele of a second gene of the series, $b^+$, controls a reaction: A $\longrightarrow$ B; (2) the mutant alleles block or impair these reactions; and (3) the substance A, at least, is produced at, or very close to, the site of the gene in the chromosome and is effectively transported along the chromosome more readily than it is transported to the homologous chromosome. As may be seen from the diagrams below, the cis-arrangement of the wild-type alleles (1) is expected on the above assumptions to give a more nearly normal action (production of substance B) than the trans-arrangement (2):

(1)

$$\frac{S \xrightarrow{a^+} A \xrightarrow{b^+} B}{S \xrightarrow[a]{} {<}A \xrightarrow[b]{} {<}B}$$

(2)

$$\frac{S \xrightarrow{a^+} A \xrightarrow{b} {<}B}{S \xrightarrow[a]{} {<}A \xrightarrow[b^+]{} {<}B}$$

A new kind of supporting evidence for this model has come from the discovery that, in the case of the bithorax pseudoallelic series, structural heterozygosity for certain chromosomal rearrangements ($R$) significantly alters the phenotype of particular trans-types towards a more extreme departure from wild-type, yet, in general, does not alter the phenotype of the cis-type. This new type of position effect, detected by comparing $R(a+)/+b$ or $a+/R(+b)$, on the one hand, with $a+/+b$ on the other, will be referred to as the "trans-vection effect." As recently described in some detail (Lewis, 1954b), the majority of X-ray-induced rearrangements which have at least one breakage point between the centromere and the locus of *bx* (a distance of over 500 "bands" of the salivary gland chromosomes) evoke the trans-vection effect; moreover, the results of the analysis of these strongly supports the hypothesis that a reduction in somatic pairing is the causative factor in modifying the phenotype of the trans-type. The trans-vection effect phenomenon is readily understandable on the above model by assuming

that a reduction in somatic pairing effectively blocks the residual transport of substance A (diagram 2, above) from its site of production in one chromosome to the corresponding site in the homologous chromosome.

The position effect which is detected by comparing the cis- and trans-type and which is the basis for defining position pseudoallelism now needs a term to distinguish it from the trans-vection effect, and will henceforth be referred to as the "cis-vection effect" (Lewis, 1954b). A variety of cis-vection and trans-vection effects are known in the bithorax case, and the remainder of this paper will be devoted to a systematic treatment of these phenomena.

## CIS-VECTION EFFECTS

Evidence for five loci in the bithorax pseudoallelic series has recently been presented (Lewis, 1954a). In order from left to right starting at locus 58.8 in the third chromosome, the loci are: bithorax (*bx*), Contrabithorax (*Cbx*), Ultrabithorax (*Ubx*), bithoraxoid (*bxd*) and postbithorax (*pbx*). The evidence was, however, incomplete in that between two pairs of genes, *Cbx* and *Ubx*, and *bxd* and *pbx*, only the wild-type crossovers had been recovered from females of the trans-type. Since then the complementary crossovers, *Cbx Ubx* and *bxd pbx*, respectively, have been obtained from females of the appropriate trans-type. Cis-vection effects have been studied by comparing all ten possible double mutant combinations in the cis- and trans-types for the mutants, $bx^3$, *Cbx*, *Ubx*, *bxd* and *pbx*. Before considering them, however, the individual mutant phenotypes will be briefly reviewed.

Three of the mutants, $bx^3$, *Ubx* and *bxd*, are spontaneous in origin and have been described in some detail before (see Bridges and Brehme, 1944; Lewis, 1951; *Ubx* was formerly designated as $bx^D$, in the former reference; as *Bxl*, in the latter; and as $bxd^D$ by Lewis, 1949). The *Cbx* and *pbx* mutants are of X-ray origin, and, as recently reported (Lewis, 1954a) are remarkable in that they appear to have been induced simultaneously. Each of these five mutant genes appears to be normal in the salivary gland chromosomes. These genes effect well-defined transformations of certain body segments or parts of body segments. At least four sharply distinct and more or less independently varying transformations of this kind have been recognized. On the basis of these, each mutant can be rather precisely described and readily distinguished from any one of the others (see table 1). Thus, the $bx^3$ homozygote has the anterior portion of the metathorax (AMT) transformed into a structure which very closely resembles the anterior portion of the mesothorax (AMS)—a transformation (Type I) which will be symbolized: AMT → AMS. The *pbx* homozygote has a second type of transformation (Type II); namely, a conversion of the posterior portion of the metathorax (PMT) into a structure very closely resembling the posterior portion of the mesothorax (PMS), or symbolically: PMT → PMS. The *bxd* homozygote also has this Type II transformation, but not quite so well developed, and, in addition, always has a thoracic-like modification of the

TABLE 1

INDIVIDUAL MUTANT PHENOTYPES OF THE BITHORAX PSEDUOALLELIC SERIES

Legend: 0 = little or no transformation; hence wild-type, or nearly so; +, ++, +++, ++++ = very slight, slight, moderate, extreme degrees, respectively, of the indicated transformation.

| Name of locus | Genotype | Type of body segment transformation | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| bithorax | $bx^3/bx^3$ | ++++ | 0 | 0 | 0 |
| postbithorax | pbx/pbx | 0 | ++++ | 0 | 0 |
| bithoraxoid | bxd/bxd | 0 | +++ | +++ | 0 |
| Contrabithorax | Cbx/+ and Cbx/Cbx | 0 | 0 | 0 | ++++ |
| Ultrabithorax | Ubx/+ | + | 0 | 0 | 0 |

first abdominal segment ($AB_1$). The latter transformation (Type III) is primarily towards a structure resembling AMT, and will be symbolized: $AB_1 \rightarrow$ AMT; however, the presence of posteriorly wing-like halteres on $AB_1$ (thus far found only in $bxd/bxd^{121}$, where $bxd^{121}$ is of X-ray origin) implies that posteriorly $AB_1$ changes towards PMS. The *Ubx/+* genotype has an extremely slight Type-I transformation (recognizable only in the haltere, whose distal segment is enlarged and more hairy on the anterior margin than the wild-type haltere). The *Ubx* homozygote is lethal in the adult stage but the larval phenotype and interactions with other mutants of the series indicate that it is phenotypically like double-mutant homozygotes between *bxd* and a *bx* mutant; *i.e.*, combines Type-I, -II, and -III transformations (the combination of Type I and Type III giving a transformation of $AB_1$ towards AMS). Whether *Ubx* differs qualitatively from such double mutant combinations is not clear. The *Cbx* homozygote, as well as the virtually identical *Cbx/+* genotype, has a fourth transformation (Type IV); namely, a reduction in the development of PMS so that it partially resembles, especially in the case of the wing, PMT—or a change in the mesothorax which may be written: PMS $\rightarrow$ PMT. Occasionally, the *Cbx* phenotype has also a reduction in AMS so that the latter begins to resemble AMT, especially in the case of the wing which becomes almost completely haltere-like.

Since the first known mutant of the above series, $bx^1$ of Bridges (see Bridges and Brehme, 1944), is highly variable and may occasionally overlap wild-type, the false impression may have arisen in some quarters that these so-called homeotic mutants are intrinsically highly variable. On the contrary, all of the above described mutant effects are surprisingly uniformly expressed, with the exception of the variability noted for the *Cbx* mutant. In no case have any of these phenotypic effects, including those of the *Cbx* mutant and the slight dominant effect of *Ubx/+*, been observed to overlap the wild-type phenotype.

With the above five mutant genes and their ten possible double mutant combinations, there are ten possible pairs of cis- and trans-types to be compared for cis-vection effects. All ten pairs have been constructed and their phenotypes are summarized in table 2 in terms of four transformation types

TABLE 2

CIS-VECTION AND TRANS-VECTION EFFECTS INVOLVING THE BITHORAX PSEUDOALLELIC SERIES (LEGEND AS IN TABLE 1)

| Group | Mutants in heterozygote | Type of heterozygote | Type of body segment transformation | | | |
|---|---|---|---|---|---|---|
| | | | I | II | III | IV |
| 1. | a. $bx^3$ and bxd | cis | 0 | 0 | 0 | 0 |
| | | trans | 0 | 0 | 0 | 0 |
| | | R-trans | 0 | 0 | 0 | 0 |
| | b. $bx^3$ and pbx | cis | 0 | 0 | 0 | 0 |
| | | trans | 0 | 0 | 0 | 0 |
| | | R(cis) | 0 | 0 | 0 | 0 |
| | | R(trans) | 0 | 0 to + | 0 | 0 |
| | c. bxd and pbx | cis | 0 | 0 | 0 | 0 |
| | | trans | 0 | +++ | 0 | 0 |
| | | R(trans) | 0 | +++ | 0 | 0 |
| 2. | a. $bx^3$ and Ubx | cis | + | 0 | 0 | 0 |
| | | trans | +++ | + | 0 | 0 |
| | | R(trans) | ++++ | + | 0 | 0 |
| | b. Ubx and bxd | cis | + | 0 | 0 | 0 |
| | | trans | + | +++ | +++ | 0 |
| | | R(trans) | + | +++ | +++ | 0 |
| | c. Ubx and pbx | cis | + | 0 | 0 | 0 |
| | | trans | + | ++++ | 0 | 0 |
| | | R(trans) | + | ++++ | 0 | 0 |
| 3. | a. $bx^3$ and Cbx | cis | 0 | 0 | 0 | ++ |
| | | trans | 0 to + | 0 | 0 | ++++ |
| | | R(trans) | 0 to + | 0 | 0 | ++++ |
| | b. Cbx and bxd | cis | 0 | 0 | 0 | ++++ |
| | | trans | 0 | 0 | 0 | ++++ |
| | | R(trans) | 0 | 0 | 0 | ++++ |
| | c. Cbx and pbx | cis | 0 | 0 | 0 | ++++ |
| | | trans | 0 | 0 to + | 0 | ++++ |
| | | R(trans) | 0 | 0 to + | 0 | ++++ |
| 4. | a. Cbx and Ubx | cis | + | 0 | 0 | + |
| | | trans | ++ | + | 0 | +++ |
| | | R(cis) | + | 0 | 0 | 0 |
| | | R(trans) | ++ | + | 0 | +++ |

described above. For the sake of systematic presentation, the results will be discussed in terms of the four groups of comparison that can be made on the basis of dominant and recessive relationships.

Group-1 comparisons involve only the recessive mutants. The cis- and trans-types for $bx^3$ and *bxd* are both wild-type in phenotype; thus, no cis-vection effect is in evidence. The cis- and trans-types for $bx^3$ and *pbx* are also wild-type; in contrast with the previous comparison, however, these genotypes can be shown to differ phenotypically by making each a structural heterozygote for chromosomal rearrangements which evoke moderate to ex-

treme trans-vection effects. As described elsewhere (Lewis, 1954b), the translocation, T(2; 3) $bw^{VDe3}$, which has the major portion of the right arm of the third chromosome reciprocally translocated to the distal portion of the right arm of the second chromosome, is such a rearrangement, and is useful to employ since it has an inseparable, dominant, variegated-brown effect. By the use of this rearrangement, it is found that the cis-type, $R(++)/bx^3\,pbx$ remains wild-type; while each of the trans-types, $R(bx^3+)/+pbx$ and $bx^3+/R(+pbx)$, occasionally has a very slight wing-like development of the posterior region of the haltere; that is, a slight Type-II transformation. Finally, the cis-type, $bxd\,pbx/++$, is wild-type; while the trans-type, $bxd+/+pbx$, has a moderate Type-II transformation, thus indicating a strong cis-vection effect.

Group-2 comparisons involve each of the three recessive mutants with the dominant *Ubx* mutant. Each of the three comparisons of this kind shows pronounced cis-vection effects. On the one hand, the three cis-types, $bx^3\,Ubx/++$, $Ubx\,bxd/++$, and $Ubx\,pbx/++$ are phenotypically indistinguishable from each other and from the single dominant mutant heterozygote, *Ubx/+*, which, as already noted, differs from wild-type only by a very slight Type-I transformation. On the other hand, $bx^3+/+Ubx$ combines a moderate Type-I (figured by Lewis, 1951) with a very slight Type-II transformation; $Ubx+/+bxd$ combines the above very slight Type-I with moderate Type-II and moderate Type-III transformations (since no haltere-like structure has been observed on $AB_1$ of this genotype, it is not possible to observe whether the very slight transformation of Type I combines in that segment with the Type III one to produce a mesothoracic-like modification of this segment): while the remaining trans-type, $Ubx+/+pbx$ combines the very slight Type-I with a very extreme Type-II transformation.

Group-3 comparisons involve each of the three recessive mutants with the dominant *Cbx* mutant. The comparison of $bx^3\,Cbx/++$ with $bx^3+/+Cbx$ reveals another type of cis-vection effect. Thus, the trans-type is like the single mutant heterozygote, *Cbx/+*; it has a well-developed Type-IV transformation. The cis-type, on the other hand, has only a slight transformation of this type. The trans-type sometimes also has a very slight Type-I transformation which the cis-type lacks. The trans-types, $Cbx+/+bxd$ and $Cbx+/+pbx$, show no striking differences from *Cbx/+*, nor from their respective cis-types; however, $Cbx+/+pbx$ appears to have the beginning of a Type-II transformation in the region of the haltere, while its cis-type is wild-type in this respect. At the same time, there are striking differences between the cis- and trans-types in each of these two latter comparisons when they are studied in the presence of a recessive, sex-linked, partial suppressor of *Cbx* (symbol, *su-Cbx*; *locus*, $30^{\pm}$; spontaneous in a stock of $v\,Bx^r$; $bxd^{51J}$, kindly supplied to the author by M. M. Green). Thus, males of the cis-types, *su-Cbx*; $Cbx\,bxd/++$, and *su-Cbx*; $Cbx\,pbx/++$ (as well as the genotypes, *su-Cbx*; *Cbx/+* and *su-Cbx*; $bx^3\,Cbx/++$) differ from wild-type only in having a very slight Type-IV transformation; that is, they have an

almost complete suppression of the dominant effect of *Cbx*. By contrast, in addition to this latter very slight transformation of Type IV, males of the trans-type, *su-Cbx*; *Cbx+/+bxd*, have a moderate Type-III, and males of the trans-type, *su-Cbx*; *Cbx+/+pbx*, have a slight Type-II transformation. Furthermore, males of the trans-type, *su-Cbx*; $bx^3+/Cbx+$, have a slight Type-I transformation as well as the very slight Type-IV one.

Finally, group-4 comparisons involve the two dominant mutants. The cis-type, *Cbx Ubx/++*, is remarkable in that there is scarcely any detectable Type-IV transformation; the alula of the wing is reduced and the wings are often slightly spread, but the phenotype otherwise is wild-type except for the typical very slight Type-I transformation characteristic of *Ubx/+*. On the other hand, the trans-type, *Cbx+/+Ubx* has a moderate transformation of Type IV (not quite so extreme as that of *Cbx/+*), a slight one of Type I (the haltere being larger and more hairy than that of *Ubx/+*), and a very slight one of Type II.

It is a general rule that cis-vection effects are very striking position effects, in that the cis- and trans-types not only differ strikingly in phenotype, but they show little or no tendency to overlap one another in phenotype over a wide range of environmental conditions. This rule applies to all of the above cis-vections shown in table 2, except that the phenotype of $R(bx^3+)/+pbx$ may overlap wild-type and hence overlap that of $R(++)/bx^3\,pbx$; however if a more complex rearrangement (with respect to structural heterozygosity of the *bx* region) than $bw^{VDe3}$ is employed then there is virtually no overlapping of these two phenotypes.

To summarize, a broad spectrum of cis-vection effects have been met with in the case of the *bx* pseudoallelic series. Both cis- and trans-types for a given pair may prove to be wild-type under perhaps all conditions ($bx^3$ and *bxd*). This case is analogous to that of miniature (*m*) and dusky (*dy*) mutants studied by Slatis and Willermet (1953) who find that *m+/+dy* and *m dy/++* are each virtually wild-type, although the latter may possibly have significantly shorter wings than the latter. The cis- and trans-types may be wild-type under normal conditions but differ phenotypically when both are made into identical structural heterozygotes ($bx^3$ and *pbx*). It becomes evident from this case that there may be no sharp line between pseudoallelism with and without the position effect phenomenon: thus, the possibility of position effect is obviously to be kept in mind in the numerous cases of pseudoallelism where both cis- and trans-types are wild-type or otherwise identical in phenotype; e.g., cases of pseudoallelism in mice (Dunn and Caspari, 1942; 1945) or cotton (see reviews of this and other cases by Stephens, 1951; and Komai, 1950). A striking cis-vection effect occurs between two recessive mutants (*bxd* and *pbx*). The latter case is analogous to the cases of lozenge, apricot-white, and vermilion pseudoalleles, already referred to. Striking differences occur in every comparison involving any one of the recessive mutants with *Ubx*; these effects are analogous to those observed with the Star-asteroid series. In comparisons involving *Cbx* unusual

relations arise. Thus, the trans-type may be mutant but not strikingly different from *Cbx*/+; yet position pseudoallelism is indicated by the strikingly different and much more nearly normal cis-type (*Cbx* and $bx^3$). Or both cis- and trans-types may be mutant and nearly, if not quite, identical; yet cis-vection effects can be revealed in the presence of a sensitizing modifier gene; *su-Cbx*, (*Cbx* and *bxd*, or *Cbx* and *pbx*). *Ubx* acts as a virtually complete suppressor of the dominant effect of *Cbx* in the case of the cis-type, but has only feeble interactions with *Cbx* in the case of the trans-type. This comparison and that involving *Cbx* and $bx^3$ parallel somewhat the Stubble-stubbloid case (Lewis, 1951); thus stubbloid acts as a complete suppressor of the dominant Stubble phenotype in the case of the cis-type but gives an extreme mutant phenotype in the case of the trans-type.

## TRANS-VECTION EFFECTS

To study trans-vection effects, the translocation, $bw^{VDe3}$, has been used to produce structural heterozygosity. This rearrangement has been combined by crossing over with all of the single mutant types except *Cbx* and most of the double mutant types (the exceptions being *Cbx Ubx*, *Cbx bxd* and *Cbx pbx*). In no case has heterozygosity for this translocation modified the phenotype of a heterozygote for a single mutant gene, and in only one case (discussed below) is the phenotype of the cis-type between two mutants modified. The manner in which certain of the trans-types are altered by heterozygosity for this translocation will be discussed by systematically considering the four groups of double mutant types already adopted. The results are also shown in table 2. In the case of Groups 1 and 2, comparisons have been made in every case between the trans-type without structural heterozygosity, on the one hand, and the two forms of the structurally heterozygous trans-type, or "*R*(trans)-type" as it is designated in table 2, on the other hand. In no case has there been any obvious difference between the two forms of an *R*(trans)-type; that is, between *R*(a+)/+b and *a*+/*R*(+*b*). Both forms of the "R(cis)-type," as the structurally heterozygous cis-type is designated, have also been constructed in all of the Group-1 and -2 cases. Again, in no case involving Groups 1 and 2 has there been an obvious phenotypic difference between such pairs; moreover, since in such cases the *R*(cis)-type does not differ from the cis-type, the R(cis)-type has been omitted from table 2 except where explicitly needed. In the case of Groups 3 and 4 of table 2, only one form of the *R*(trans)-type or *R*(cis)-type has been constructed; and only where the phenotype of the *R*(cis)-type was different from that of the cis-type is it included in the table.

Among the Group-1 comparisons of trans-types with and without structural heterozygosity, only the one involving $bx^3$ and *pbx* shows a trans-vection effect. In this case, *R*($bx^3$+)/+*pbx* occasionally has a very slight Type-II transformation in contrast to the wild-type phenotype of $bx^3$+/+*pbx*.

Among the group-2 comparisons, the only trans-vection effect yet detected involves $bx^3$ and *Ubx*. In this case, *R*($bx^3$+)/+*Ubx* has an extreme Type-I transformation compared to a moderate Type I found in $bx^3$+/+*Ubx*.

Among the group-3 comparisons, no obvious trans-vection effects are present. However, preliminary studies utilizing the above-described modifier gene, su-Cbx, have shown that *su-Cbx*; $R(bx^3+)/+Cbx$ males have a significantly more extreme Type-I transformation than su-Cbx; $bx^3+/+Cbx$ males.

Among the Group-4 comparisons, there is one trans-vection effect and it is of a new type. Whereas, in all other examples of this phenomenon, it is only the trans-type that is modified by the appropriate kind of structural heterozygosity, in this case it is only the cis-type which is modified. Thus, the comparison of the trans-type and the R(trans)-type for *Cbx* and *Ubx* reveals no obvious phenotypic difference. On the other hand, the *R*(cis)-type is more nearly wild-type than the cis-type; that is, *R*(++)/*Cbx Ubx* lacks the very slight Type-IV transformation characteristic of ++/*Cbx Ubx*. The latter result has, moreover, been verified for a number of different *R*'s besides $bw^{VDe3}$.

It is a general rule that the phenotypic differences involved in the recognition of the trans-vection effects are relatively slight ones. Maximum sensitivity for the detection of such differences requires attention to environmental conditions: constant temperature (25°C) and sufficient food. Combinations of two different rearrangements or single rearrangements more complex than $bw^{VDe3}$, for example, have been used effectively to increase such differences, as in the case of $bx^3$ and *pbx*, noted above. Such techniques, as well as the use of sensitizing modifier genes, may help to reveal trans-vection effects in the cases where none have yet been detected. Although isogenic stocks have not been employed, an approach to complete control over the genetic background has been made in most of the observed trans-vection effects by utilizing a variety of stocks of each mutant type (containing different substitutions with respect to closely linked marker genes), and by utilizing different chromosomal rearrangements of independent origin. Such techniques have actually failed to reveal differences traceable to modifier genes; that is, the results are reproducible under a wide variety of genetic backgrounds and with different rearrangements.

To summarize, trans-vection effects have been detected between $bx^3$ and *Ubx*, $bx^3$ and *pbx*, $bx^3$ and *Cbx* (if sensitized by the presence of *su-Cbx*), and *Cbx* and *Ubx*, as well as between $bx^{34e}$ and *Ubx* (as reported in a previous study—Lewis, 1954b). Thus, such effects are not dependent upon the presence of some particular mutant type. The mutants, *Cbx* and *Ubx*, give a unique result in that a phenotypic difference arises only between the *R*(cis)-type and the cis-type; while in all of the other cases, it arises only between the *R*(trans)-type and the trans-type.

## DISCUSSION

An attempt will be made below to construct a model of gene-controlled reactions in the case of the bithorax pseudoallelic series which will (1) take account of the principal mutant transformations, (2) explain as simply

as possible the observed cis-vection and trans-vection effects, and (3) give some picture of how the genes control the wild-type segmentation pattern.

In a previous report (Lewis, 1951) it was pointed out that the position effects between $bx^3$ and *Ubx*, and *Ubx* and *bxd* (cis-vection effects, in the newer terminology), can be simply interpreted on the basis of three successive, gene-controlled reactions occurring at the chromosomal level. The order of control could be postulated to be either: $bx^+ - Ubx^+ - bxd^+$ or $Ubx^+ - bx^+ - bxd^+$. In addition to the finding that this scheme satisfactorily accounted for the phenotype of a great many genotypes, it received independent support from studies of chromosomal rearrangements having one breakage point within the pseudoallelic series itself. Thus, rearrangements which apparently separate $bx^+$ and $Ubx^+$ on the one hand from $bxd^+$, on the other, were found to have no detectable change in the action of the $bx^+$ and $Ubx^+$ genes but were found to act like extreme mutant changes in the action of $bxd^+$. For example, the homozygote for the transposition, $bxd^{100}$ (which has $bx^+$ and probably $Cbx^+$ and $Ubx^+$ transposed to the left arm of the third chromosome) has extreme transformations of the *bxd* type (Type II and Type III), but not of the *bx* type (Type I). On the above scheme the trans-type between $bx^3$ and *bxd* might also be expected to give the *bxd* types of transformations; while the cis-type would be expected to be wild-type. Although the trans-type as well as the cis-type in the case of $bx^3$ and *bxd* is wild-type, this was not regarded as necessarily inconsistent with the above scheme since both $bx^3$ and *bxd* could be regarded as intermediate alleles of their respective loci and, therefore, as mutants which would only partially block their respective gene-controlled reactions. The latter interpretation was especially plausible since (1) *bx*-like mutants of X-ray origin, which are more extreme than $bx^3$, give strong Type-II and Type-III transformations in the trans-types with *bxd*; and (2) extreme *bxd*-like mutants of X-ray origin, such as $bxd^{100}$, give a very slight Type-II transformation in the trans-type with $bx^3$. In neither of these cases could the assumption of a cis-vection be tested, since the complication of chromosomal rearrangements associated with the X-ray induced changes prevented the recovery of cis-types.

The new findings reported here concerning the *pbx* mutant help to clarify the above findings regarding the Type-II transformation, and permit an extension of the above scheme to include the $pbx^+$ gene. Thus, the Type-II transformations which have just been discussed, as well as those which characterize the trans-types between *pbx* on the one hand, and $bx^3$, *Ubx*, or *bxd*, on the other, are all readily accounted for if it is assumed that (1) $pbx^+$ controls a reaction subsequent to that of $bxd^+$, and (2) a reduction in the concentration of the gene product of $pbx^+$ leads to the Type-II transformation. This gives the scheme "Scheme 1" (see Figure 1) for the sequence of gene-controlled reactions. Although the order of control of reactions by $bxd^+$, $pbx^+$, and either $bx^+$ or $Ubx^+$ is the same as the gene order in the chromosome, the order of control with respect to $bx^+$ and $Ubx^+$ cannot be deduced from the data and is arbitrarily assumed to be the same as that in the chromosome to simplify the discussion of this scheme.

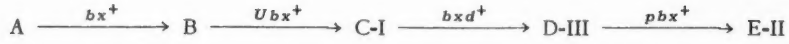


FIGURE 1

The omission of $Cbx^+$, whose locus in the chromosome is between those of *bx* and *Ubx*, is intentional, since as discussed below its role in such a scheme is not clear. In the above notation the roman numeral after the symbol of the gene product identifies the type of transformation controlled by that product. More exactly, a reduction in the relative concentration of substance C-I is postulated to lead to a transformation of Type I; of D-III to one of Type III; and of E-II to one of Type II. The substances, C-I and D-III, at least, are assumed to be produced in sufficient concentration to act not only as substrates for the appropriate successive step in the chain, but also as agents which somehow ultimately direct the control of specific physiological processes. The intermediate substances may be thought of as enzymes, as enzyme percursors, or more likely as products of enzymatic activity, that activity in the latter case coming either directly from the gene or from an enzyme closely held to the gene. The essential requirement for the interpretation of the observed cis-vection and trans-vection effects is that these substances be produced at, or very close to, the site of the genes in the chromosome, rather than at other points in the cell.

Specifically, the Type-II transformations discussed above are accountable for on Scheme 1 in the following way. In the case of the trans-type, $bx^3+/+bxd^{100}$, the $bx^3$ mutant is assumed to reduce relatively slightly the concentration of B, which then leads indirectly to a relatively slight reduction in E-II; in the other chromosome, the separation of $bx^+$ and $pbx^+$ is expected to result in a relatively extreme reduction in the concentration of E-II; the observed very slight Type-II transformation is thus accounted for. In the case of $bx^3+/+pbx$, $bx^3$ is assumed to act as in the preceding case, while a relatively extreme reduction in E-II in the other chromosome is postulated to result from a blocking of the final reaction by the *pbx* mutant; the observed result, that a very slight Type-II transformation arises in this case only if there is at the same time structural heterozygosity, will be discussed below. In the case of $Ubx+/+pbx$, the *Ubx* mutant is assumed to cause a relatively extreme reduction in C-I and in turn of E-II, while the *pbx* would act as in the previous case; the observed extreme Type-II transformation is thus accounted for. Finally, in the case of $bxd+/+pbx$, the *bxd* mutant is assumed to cause a moderate reduction of D-III and therefore in turn of E-II; while the *pbx* mutant would act as before; the observed moderate Type-II transformation is thus accounted for. In the case of the three respective cis-types, $bx^3\,pbx/++$, $Ubx\,pbx/++$, and $bxd\,pbx/++$, the chromosome carrying the wild-type alleles of the pseudoallelic genes is assumed to produce its normal quota of substance E-II and this quota is assumed to be sufficient to prevent the Type-II transformation, even though the other chromosome might fail to produce any E-II; the observed failure of the cis-types to show a Type-II transformation is thus accounted for. The remain-

ing cis-vection effect comparisons involving the four loci shown in Scheme 1 are those involving the following pairs: *bx*$^3$ and *Ubx*, *bx*$^3$ and *bxd*, and *Ubx* and *bxd*. As already noted these cases have been discussed elsewhere (Lewis, *loc. cit.*) in detail in terms of a model including the first three steps of Scheme 1. The only change that Scheme 1 introduces is to interpret Type-II transformations as being controlled not by the product of the *bxd*$^+$ gene, but by that of *pbx*$^+$. In the case of *bx*$^3$+/+*bxd*, it remains necessary to postulate a threshold effect to explain its wild-type phenotype; in other words, the reduction in amount of substances D-III and E-II which is expected in this genotype on Scheme-1 is postulated to be below the threshold required to give a detectable mutant phenotype.

The application of the sequential reaction type of interpretation to trans-vection effects has been discussed elsewhere (Lewis, 1954b) and will only be briefly considered here. In the case of *bx*$^3$+/+*Ubx*, for example, the *Ubx*$^+$ gene is assumed on Scheme-1 to receive some of its substrate B from the action of the *bx*$^3$ gene and some from diffusion of B from its site of production in the homologous chromosome; while in the presence of structural heterozygosity, the *Ubx*$^+$ gene is assumed to be deprived (relatively speaking) of this latter source of B as the result of reduced somatic pairing of the homologous chromosomes at that site. A similar argument would apply to the remaining trans-vection effect observed in Groups 1 and 2 of table 2; namely, that involving *bx*$^3$ and *pbx*; here, however, the substance(s) involved might be any of the intermediates: B, C-II and/or D-III.

It is important to consider how, conversely, the production of substances C-I, D-III and E-II would be expected to control the development of the wild-type segmentation pattern. This should provide a test of the utility of Scheme-1 and an aid to the understanding of the mutant phenotypes. Thus, the production of substances C-I and E-II is postulated to cause the wild-type metathoracic segment, to advance from a primitive level of developmental determination, which will be designated as a mesothoracic-like level, "L-ms," to a metathoracic-like level, or "L-mt." On evolutionary grounds, L-ms is more primitive than L-mt, since the Diptera almost certainly evolved from four-winged ancestors. A reduction in concentration of C-I or E-II would be expected on this basis to cause AMT or PMT, respectively, to tend to remain at the level, L-ms, which agrees with the respective definitions of the Type-I and Type-III transformations.

The production of substance D-III is postulated to cause the first abdominal segment of the wild-type organism to change from a primitive level, which again will be designated L-ms, to a first-abdominal level, or "L-ab$_1$." On evolutionary grounds, L-ms is probably more primitive than L-ab, since the ancestors of the insects certainly had legs on the abdominal segments, and the immature stages of many insects bear ventral abdominal appendages. In an earlier discussion of these levels of developmental determination (Lewis, 1951) it was assumed that this product of the *bxd*$^+$ gene (D-III) causes the first abdominal segment to change in a way that would be the exact reverse of the Type-III transformation (AB$_1$ $\rightarrow$ MT); that is, causes

change: L-mt → L-ab$_1$. This earlier assumption is unsatisfactory on evolutionary grounds and does not give a clear picture of the way in which development of this segment would take place in certain genotypes; it will therefore be discarded in favor of the above assumption that the production of D-III leads to the change: L-ms → L-ab$_1$. In other words, the first abdominal segment is postulated to develop according to two alternate pathways, rather than according to two successive pathways (namely, L-ms → L-mt → L-ab$_1$), as formerly assumed. The alternate pathways and their specific controlling substances will be designated Scheme-2, and may be represented as follows:

$$\text{L-mt} \xleftarrow{\text{C-I + E-II}} \text{L-ms} \xrightarrow{\text{D-III}} \text{L-ab}_1$$

This scheme gives for the first time an adequate explanation of the development of AB$_1$ in the principal mutant types as well as in the wild-type organism. The following examples illustrate the way in which Scheme-2 is assumed to apply in such cases (see table 1 for the observed effects). Thus, the *bx*$^3$ homozygote possesses a sufficient quantity of substance D-III to direct AB$_1$ along the pathway to L-ab$_1$. The *bxd* homozygote lacks a sufficient quantity of D-III to direct the segment along this latter pathway, and lacks sufficient E-II to direct the posterior portion towards PMT, so that this portion should remain at level, L-ms; however, it has sufficient C-I to direct the anterior portion towards the level, L-mt. Finally, homozygotes for double mutants between *bx* and *bxd* mutants, or for *Ubx*, lack sufficient quantities of all three substances, so that AB$_1$ should remain at the level, L-ms.

The remaining segment of the wild-type organism to be considered in connection with Schemes 1 and 2 is the mesothorax. It is presumed that Scheme 1 does not effectively come into play during the development of this segment, perhaps because of an anterior-posterior gradient in the distribution of the initial substrate A. Thus, lack of sufficient quantities of substances C-I, D-III and E-II would leave MS at its primitive level of development, L-ms.

Scheme-1 has been found to account adequately for all of the genotypes constructed to date involving the *bx*$^3$, *Ubx*, *bxd* and *pbx* mutants, except for one case: *bx*$^3$++/+*Ubx bxd*. (Some 56 such genotypes among a possible total of 136 have been constructed, the remaining ones involving chiefly triple and quadruple mutant combinations, which have not yet been synthesized.) This exceptional case, omitted above since it is a triple mutant heterozygote, has a more extreme Type-I, and a less extreme Type-II transformation than does the genotype, *bx*$^3$++/+*Ubx*+. On Scheme-1 it would be expected that the former genotype would, if it did so at all, differ from the latter by having a less extreme Type-I transformation, as a result of the possibility of an accumulation of substance C-I, and a more extreme Type-II transformation, as a result of two blocks along the pathway to E-II in the

*+ Ubx bxd* chromosome, compared to only one in the *+ Ubx +* chromosome. For in other cases, just such predictions are verified; for example, $bx^{3}$ *+ bxd/*$bx^{34}$ *++* has a less extreme Type-I transformation than $bx^{3}$ *++/* $bx^{34e}$ *++* (where $bx^{34e}$ is an intermediate *bx* allele); and *Ubx bxd/+ bxd* has a more extreme Type-II transformation than does *Ubx +/+ bxd*.

The above difficulty in interpreting one exceptional genotype on Scheme-1 has not been resolved and indicates that at least one more variable is needed, if it is to remain a valid working hypothesis. There is such a variable in the case of substance B, since it has not been assigned a specific function (other than as substrate for the $Ubx^{+}$ gene). There is also the question of the role of $Cbx^{+}$ in Scheme 1, since this gene and its product constitute an additional variable, yet to be considered. But just what role $Cbx^{+}$ may play is not clear. Many of the interactions of the *Cbx* mutant with other mutants of the series indicate that $Cbx^{+}$ may control an additional step near the beginning of the reaction sequence; while its dominant effect may be attributed to an accumulation of some product such as B or C-I which would begin to cause MS to develop towards MT. However, since the *Cbx* transformation (Type IV) is the inverse of those of Type I and Type II, the possibility of complicated interactions at a physiological level between the numerous hypothetical gene products involved in such transformations becomes an acute one.

The unique finding of a trans-vection effect by comparing cis- and *R*(cis)-types involving *Cbx* and *Ubx* can be formally reconciled with the other trans-vection effects, even though the role of *Cbx* in Scheme-1 is not clear. Thus, it may be that in *Cbx Ubx/++* a higher concentration of substance B reaches the *Ubx* gene than does so in *R(++)/Cbx Ubx*, where the homologous chromosomes are relatively farther apart; this would mean more of substance C-I, or a more nearly normal Type-I transformation, in the cis-type than in the *R*(cis)-type; in turn this would lead, paradoxically, to the observed result that the cis-type has a more extreme Type-IV transformation than the *R*(cis)-type, since the available evidence suggests that the more extreme the Type-I change the less extreme the Type-IV change.

## CONCLUSIONS

It is concluded that there is an ordered complexity to the variety of cis-vection and trans-vection effects, as well as individual mutant effects, in the case of the bithorax pseudoallelic series. The "functional" interpretation has to explain such results in terms of a concept of a single functional unit of probably considerable complexity—analogous to the old concept of the gene as deduced from the behavior of complex "multiple allelic" series. The "genetic" interpretation explains such results in terms of a working hypothesis in which each of the component genes of the series acts as a functional unit in the modern sense—namely, as an agent controlling a single, specific reaction.

SUMMARY

Contrasting interpretations of position pseudoallelism are discussed, and the types of position effect which characterize this phenomenon are illustrated, with special reference to the case of the bithorax series of five pseudoallelic loci in *Drosophila melanogaster*. Most of the results in this latter case can be simply interpreted on the basis of a chain of gene-controlled reactions in which each intermediate substance is postulated to act in a dual capacity: as substrate for the succeeding reaction and as a determiner of a specific physiological process. The model also gives a consistent picture of the way in which different levels of developmental determination may be controlled by the different genes of the series.

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# STRUCTURAL AND FUNCTIONAL BASES FOR THE ACTION OF THE *A* ALLELES IN MAIZE*

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In view of the current status of our understanding of the gene it is not surprising that there exist conflicting viewpoints and interpretations regarding the nature and action of the so-called pseudoalleles which may be considered to represent a higher level of organization and complexity. The studies on crossing over which have been pursued in the analysis of all such cases clearly establish the separability, but not necessarily the individuality, of the members of such clusters. As defined in the classical sense, therefore, they are genes, though it should be obvious that this does not provide a basis for any kind of picture regarding either their structural attributes or their spatial orientation.

At the present time there are two concepts regarding the structural and functional bases for such closely linked genes and, as might have been anticipated, they correspond to the differing interpretations held for genes before it was discovered that clusters of similarly acting genes are of rather common occurrence.

According to the first of these the individual members of such clusters are discrete, localized units, though in order to account for position effects in certain instances, they have been considered to be functionally interdependent. Since it is difficult, on this basis, to explain the fact that mutant forms of the members of such gene clusters determine qualitatively similar, though not identical, deviations from the phenotypic norm, it is assumed that the member genes are somehow related in origin. According to this interpretation, which is the more widely accepted one, the close linkage between genes with similar effects is ascribable to their origin, from an ancestral locus, by some kind of intrachromosomal duplication. The hypothesis need not be restricted to the duplication of what we may think of as individual member genes since there is no evidence from these cases which would eliminate the possibility that larger segments are involved in the duplication. Actually, the latter possibility may not be discounted since there is incontrovertible cytological evidence for the existence of pattern repeats (Bridges, 1935; Metz, 1947) and since these latter are, in the words of Metz, "the ones we know are duplications."

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[1]This paper is a report of research conducted in the Botany Department of the University of Illinois.

According to an alternative viewpoint, espoused principally by Goldschmidt (1950, 1951, 1954), the close linkage between genes with similar effects is explained, without the requirement for duplication, if it is argued that the mutant forms of clusters of similarly acting genes represent modifications or impairments at different loci within a chromosome segment, the whole of which is concerned with the development of a specific normal phenotype. Thus, without any modification of a previous hypothesis, Goldschmidt finds it possible, on the structural level, to explain the close linkage between what appear to be determinants with related phenotypic effects, and on the action level, with emphasis on the segment as a whole as the functional unit, to account for the characteristic type of position effect which is associated with most of the cases known in Drosophila. In retrospect, it is surprising that adherence to the concept of the non-localized determinant, as Goldschmidt visualizes it, did not lead directly to the prediction that pseudoallelism would be found to be of common occurrence.

Almost all of the genetic evidence concerning gene clusters may be satisfactorily explained on either of these hypotheses and no doubt both schemes may be modified to accommodate certain exceptions. Because of its apparent greater simplicity the hypothesis based on segments of mutant action may appear to be favored since it explains the facts without the assumption of additional units and does not require an interaction phenomenon between discrete units to explain the position effects. However, simplicity is a requirement in a model only to the extent that it is consistent with *all* of the facts and in the cases in question there is a body of information, which has been all but ignored by Goldschmidt, suggesting that duplication of chromosomal segments is an important aspect of the pseudoalleles in Drosophila. Thus the Star-asteroid, bithorax-bithoraxoid, white-apricot (Lewis, 1945, 1951, 1952), and vermilion (Green, 1954) pseudoalleles have all been associated, in varying degrees, with doublets, or what are regarded as single band repeats, in the salivary gland chromosomes. Admittedly, due to the lack of pattern in this type of banding, it is impossible to conclude with certainty that these doublets represent duplications, but the probability that they do is enhanced by the association which occurs along their edges and which has been considered as evidence of pairing. It is probable, therefore, that intrachromosomal duplication is a characteristic cytological adjunct of pseudoallelism in Drosophila, and it may be anticipated that further analysis of these cases will have significance for the evolution of gene systems and in particular for changes in these systems which lead to increased complexity.

What is the likelihood of obtaining corresponding evidence of the association of duplication with clusters of similarly acting genes in other organisms? Unfortunately, it is impossible to obtain direct cytological evidence for intrachromosomal duplications of small or even relatively large magnitude in the higher plants, including maize. May we not expect, however, that in some instances the members of the duplication, if such it be, will have retained synaptic equivalence and, as in the case of the Bar duplication

(Sturtevant, 1925), that certain exceptional derivatives following crossing over within the cluster in obliquely synapsed strands may be taken as evidence of duplication? This expectation may be realized only if the duplication in question is of the direct or serial type since it would be expected that the special crossover derivatives which originate from reverse repeats would not be included in functional gametophytes. In this connection it is discouraging to note that the adjacent pattern repeats which have become established in Drosophila (Bridges, 1935) and in species of Sciara (Metz, 1947) are of the reverse type exclusively. Metz has argued that adjacent repeats of the serial type would be eliminated in nature because of their unstable character. Likewise, in Drosophila, where exceptional crossover derivatives of the type discussed above have not been obtained in the studies of the pseudoalleles, it has been concluded that the associated doublets are of reverse or inverted type (Lewis, 1945). The purpose of this paper is to bring together the genetic evidence bearing on the duplication basis for the $A^b$ "allele" in maize.

## THE ACTION OF $A^b$ AND ITS DERIVATIVES

In the following account of the *A* alleles and their action only those facts which are pertinent to the present discussion are reviewed. The various forms of the *A* gene control the production of varying amounts of anthocyanin pigment in the aleurone layer of the endosperm and in certain vegetative tissues. The phenotype may range from deep purple, as in the presence of *A*, the type allele, to the complete absence of anthocyanin, typical of plants carrying the recessive *a* allele. In the vegetative tissues a decrease in anthocyanin is associated with the appearance of brown pigment so that plants carrying alleles of intermediate action are characterized by mixtures of both purple and brown pigments (Laughnan, 1948, 1950). In addition the *A* alleles affect the character of the pigment which is deposited in the walls of pericarp cells provided that *P*, the basic factor for pericarp pigmentation, is present; depending on the particular *A* allele this tissue may be red or brown, but so far only the type allele, *A*, is known to produce the red phenotype. Aside from their association with the kind of phenotype which permits the easy recognition of slight differences, the special advantage of the *A* series for genetic studies lies in the divergent action of a number of its alleles (Laughnan, 1948). Curiously, those alleles whose action is non-linear, or to put it another way, is not predictable on the basis of a uni-dimensional scheme of primary effects, are either of South American extraction or trace their origin, through mutation, to such alleles. Thus, $A^b$, which was first described in a stock from Ecuador (Emerson and Anderson, 1932) would be classed as an allele of lesser effect than *A* on the basis of its effect on plant pigmentation. Yet $A^b/A$ heterozygotes have brown pericarps, indicating a dominance of $A^b$ in this regard. Moreover, the dilute mutants (designated $A^d$) which are derived from $A^b$ retain this dominance in regard to pericarp effect even though the known intermediate alleles of North American extraction, all of which have a brown pericarp

effect, are recessive to *A* in this regard. Likewise, $a^p$, which was obtained in a stock from Peru and was described by Emerson and Anderson as having a pale aleurone and plant phenotype, is dominant to *A* in pericarp effect. It is significant for later discussion to note that $a^p$ produces a somewhat weaker plant and aleurone phenotype than $A^d$.

The mutant derivatives of $A^b$ provide further evidence of non-linear action. On a uni-dimensional model it is expected that increasing doses of alleles with less than type effect should lead to corresponding increments in phenotypic effect. In the cases of the intermediate alleles of North American origin, this expectation is realized, but it does not hold for $A^d$ and $a^p$. Added doses of these alleles have no observable effect on the phenotype. Moreover, when these alleles are compounded with alleles of stronger type effect, they detract from, rather than add to, the phenotypic effect of the latter. This phenomenon, which, for lack of an adequate explanation, has been called competition, is most pronounced in the case of $a^b$ which was obtained as a two-step mutation from $A^b$ (Stadler and Laughnan, unpublished). In regard to its inherent capacity to produce pigment, this allele is the weakest-acting of all known alleles with positive effect. Special attention is given it here because it figures in the crossing over studies which will be discussed.

The recessive *a* allele employed extensively in the studies reported here is ordinarily quite stable. However, in the presence of the *Dt* gene it undergoes frequent reversions to alleles of higher level (Rhoades, 1938, 1941). While this has been considered a classical example of genetic control of gene mutation, McClintock (1950) suggests that the reversions which are observed are ascribable to changes in an inhibited state of *A*, controlled by a system similar to the dissociator-activator mechanism which she describes (1951). For the purpose of the experiments to be discussed it makes little difference which interpretation is adopted since the mutability of the recessive *a* allele is employed primarily as a technical aid in the recognition of exceptional types of crossover derivatives.

## STRUCTURAL COMPLEXITY AT THE *A* LOCUS

A number of cases are recorded of multiple allelic series whose member alleles produce something other than simple quantitative differences. Explanations of the action of alleles with divergent action have usually been based on the assumption of structural simplicity of the gene and have therefore required more elaborate arguments on the functional level (Wright, 1941, 1941a; Stern, 1943). Thus it has been necessary to assume that certain alleles may govern more than one reaction with qualitative differences between alleles held responsible for certain non-linear effects; or that a single allele may possess two or more facets of action with respect to a single reaction which it controls, and that these may vary independently for different alleles. While there is no evidence opposing such schemes it is sobering to note that they are sufficiently complicated to accommodate almost any data and will not be subject to critical test until the genes involved have

been associated with specific reactions. It may be more rewarding to revise somewhat our concept of the gene and to entertain the viewpoint that at least some of the anomalous aspects of such series are ascribable to complex gene structure, since this assumption is subject to test by a well established genetic procedure.

The results of extensive studies of this type indicate that the $A^b$ allele is compound, having components which are separable by crossing over. To summarize, from $A^b/a$ heterozygotes which may be marked genetically in various ways (table 1), exceptional gametes carrying the dilute-acting $A^d$

TABLE 1

FREQUENCIES OF OCCURRENCE OF $A^d$ DERIVATIVES OF BOTH TYPES FROM $A^b/a$ INDIVIDUALS CARRYING VARIOUS COMBINATIONS OF THE MARKERS INDICATED


| $A^b$ Gametes Tested | $A^d$ Cases | | |
|---|---|---|---|
| | Total | Crossover | Non-crossover |
| 180,500 | 135 | 124 | 11 |

allele occur with low frequency. In earlier experiments these had passed as mutations, (Stadler, 1941), but it is apparent from the later studies (Laughnan, 1949, 1952) that their occurrence is associated with crossing over. Thus, all but eight per cent of these derivatives are recombinants for the distal or rightmost marker genes. Moreover, when the closely-linked $sh_2$ (shrunken endosperm) factor is introduced as a distal marker, it is found that the crossover event which isolates $A^d$ is localized in the $A^b$ region within a segment less than one unit in length. It was concluded that this special crossover occurs between the members of an $A^b$ complex, the components of which have been designated ($\alpha$) and ($\beta$) (Laughnan, 1949). $A^d$ is considered synonymous with alpha, the left-most or proximal component, since the $A^d$-bearing strands carry predominantly the recombinant allele of the distal marker gene.

It would be predicted that beta, the distal component, should be isolated by crossing over with a frequency equal to that of the $A^d$ occurrences. While reciprocal crossovers with exceptional phenotype were not identified in the initial experiments this is not a convincing argument against their occurrence since, to account for the purple phenotype of $A^b$, it is most reasonable to consider that beta has a purple effect. Under these circumstances the beta derivatives would be indistinguishable from $A^b$ in aleurone phenotype and would not be recognizable as an exceptional class on these ears. Experiments now in progress indicate that the beta component is isolable by crossing over and is recognizable, under special circumstances, on the basis of a changed pericarp phenotype.

## THE EVIDENCE FOR DUPLICATION

What is the significance of these separable units of action at the *A* locus? A consideration of the effects of α and β on pigmentation in various tissues leads to the conclusion that while they are not identical in action they have much in common. It hardly seems plausible that the close linkage (less than 0.1 of a unit) between these genes is wholly fortuitous. If it is considered that α and β are members of a duplication, their close linkage as well as similar phenotypic effects would be explained. In the absence of critical cytological analysis, which is not feasible in this case, the opportunity to test this hypothesis genetically depends upon whether the member segments of the postulated duplication have retained some degree of homology and synaptic equivalence.

Special analyses of the $A^d$ derivatives from $A^b/a$ individuals indicate that while they are similar with respect to the level of pigmentation which they determine they differ markedly in regard to mutability in the presence of *Dt* (Laughnan, 1950a, 1952). Although most of the $A^d$ alleles are stable, from five to ten per cent of these derivatives exhibit a high rate of reversion similar to the *Dt*-induced reversions of recessive *a*, except that in the case of the mutable $A^d$ (hereafter designated $A^d$-m), as expected, the reversion areas occur on a pale rather than a colorless background. Moreover, in the cases of the several $A^d$-m alleles which have been tested, the mutability is controlled by *Dt*. Since $A^d$-m alleles originate only from $A^b/a$ individuals ($A^b/A^b$ plants have yielded only stable alleles designated $A^d$-s) and since their occurrences in all cases have been associated with crossing over it may be concluded that $A^d$-m is itself a compound "locus," having as its proximal component the α or dilute-acting allele, with *a*, the rightmost or distal component, responsible for the mutability with *Dt*. Additional evidence in support of this interpretation comes from studies of the mutational behavior of the $A^d$-m and $A^d$-s alleles. Both of these mutate to a null *a* form associated with colorless phenotype. However, the *a* derivatives from $A^d$-m are themselves mutable whereas those from $A^d$-s are stable.

The complex which is argued for $A^d$-m is expected to result when *a* is paired with $A^b$ in such a way that it lies to the right of the rare crossover which separates α from β. In terms of its components the $A^d$-m allele may be designated $\alpha_a$ whereas the $A^d$-s allele is simply α. The facts are explained in simplest fashion if it is assumed that α and β lie in adjacent segments of a duplication whose members retain synaptic equivalence. On this interpretation a crossover break between α and β may result in either a mutable or a stable $A^d$ depending on a variable pairing pattern involving the *a*-bearing segment (figure 1).

Somewhat analogous experiments involving $A^b/a^b$ heterozygotes also lead to the conclusion that $A^b$ is a duplication (Laughnan, 1951, 1952a). In this case, the strong competitive action of $a^b$, referred to previously, is employed as a technical aid in distinguishing two levels of phenotypic effect among the crossover derivatives. Thus, from *T* $A^b/a^b$ *et* individuals, suitably marked with a translocation and the gene for etched endosperm, dilute


FIGURE 1. Scheme to account for the origin of the $A^d$-s (stable) and the $A^d$-m (mutable) derivatives from $A^b/a$ heterozygotes on the basis of crossing over within the complex under conditions of a variable pattern of synapsis. The pale effect is determined by α whereas mutability resides in the *a* component.

derivatives, carrying the *T* and *et* markers, are obtained with low frequency. About half of these crossover derivatives have a phenotype identical with that of the $A^d$ derivatives from $A^b/a$ plants. The remainder are associated with a reduced phenotype similar to that which is known for the $A^d/a^b$ heterozygote. Derivatives of the latter type, tentatively designated $A^f$ (faint), have never been observed among the progeny of $A^b/a$ individuals. It may be concluded that $A^f$ is a complex of the type $\alpha a^b$ and that the reduced phenotype of $A^f$, as compared with $A^d$, is a result of the competitive effect of the now closely linked $a^b$ component. Again, on the assumption that α and β are members of a duplication, it is possible to account for these results in terms of crossover events within the α-β complex following variable synaptic patterns in sporocytes of the parent $A^b/a^b$ individuals (figure 2). This requires the assumption that α and β are homologous or lie in homologous segments which are members of a duplication.

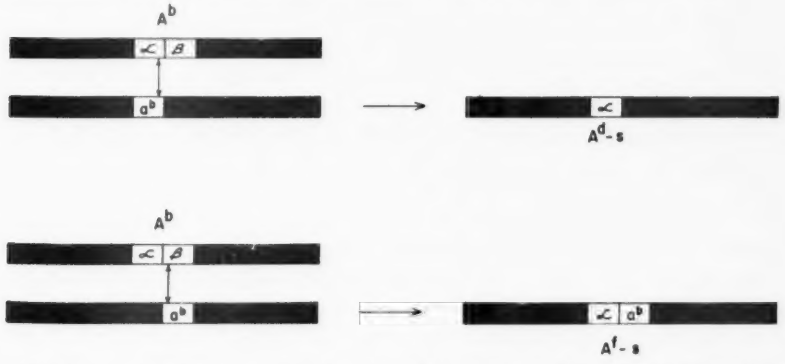


FIGURE 2. Scheme to account for the origin of the stable $A^d$ (dilute) and $A^f$ (faint) derivatives from $A^b/a^b$ as a result of crossing over within the complex.

More critical evidence favoring the duplication hypothesis is available from experiments involving $A^b/A^b$ individuals (Laughnan, 1952a). If duplication is not the basis for the separable components of $A^b$, only a regular, opposite synaptic pattern is expected at this locus in the homozygote and only those $A^d$ derivatives whose occurrence is not associated with crossing over are expected from this genotype. Fortunately, under ordinary circumstances these represent a sufficiently small proportion of all $A^d$ cases from the heterozygote to permit an efficient comparison with sib $A^b/A^b$ individuals as sources of $A^d$ exceptions. The results of such an experiment are summarized in table 2. The frequency of occurrence of $A^d$ cases known not to be associated with crossing over may be estimated among the progenies of marked $A^b/a$ individuals (table 2, row 3). This is the frequency of $A^d$ cases which is predicted to occur among gametes of $A^b/A^b$ plants if crossing over is not involved in their origin. However, $A^d$ occurrences are more than six times as frequent as expected on this basis (table 2, row 1);

TABLE 2

FREQUENCIES OF OCCURRENCE OF $A^d$ AMONG GAMETES FROM $A^b/A^b$ AND $A^b/a$ INDIVIDUALS

| | Source | $A^b$ Gametes tested | $A^d$ Cases | | Rate per million gametes |
|---|---|---|---|---|---|
| | | | Totals | Frequency ($\times 10^{-4}$) | |
| SIBS | $A^b/A^b$ | 77,940 | 30 | $3.85 \pm 0.70$ | 385 |
| SIBS | $A^b/a$ | 50,159 | 44 | $8.77 \pm 1.32$ | 877 |
| | Non-crossover cases from $A^b/a$ | 173,833 | 10 | $0.58 \pm 0.18$ | 58 |

they are, in fact, more than half as frequent as the same type of case from $A^b/a$ plants (table 2, row 2). Although this evidence is indirect, it strongly suggests that most of the $A^d$ derivatives from $A^b/A^b$ plants arise as a result of crossing over.

Tests of plants carrying suitable marker genes provide direct evidence for the crossover origin of the $A^d$ allele from homozygous $A^b$ individuals. In the first of these experiments the *lg* (liguleless leaf) factor was employed as a proximal marker, while *et* (etched endosperm) was used to define a segment to the right of $A^b$. These markers are so distant from $A^b$ (33 and 13 units, respectively) that 42 per cent of all the $A^d$ derivatives are expected to be recombinants for them even in the complete absence of association between the occurrence of the $A^d$ type and crossing over within the $A^b$ complex. Nevertheless, sufficient numbers of $A^d$ cases have been obtained and analyzed to prove conclusively that they arise, at least in part and probably in the main, as a result of crossing over within the complex in $A^b/A^b$ individuals (table 3, row 1).

In other experiments, the heterozygous condition of T 2-3d, a reciprocal translocation having its point of interchange approximately six units proximal to $A^b$, was employed in place of the *lg* factor in the hope that the origin of $A^d$ types from $A^b$ homozygotes might, with less technical difficulty, be attributed to crossing over. However, in this particular background, the

TABLE 3

SUMMARY OF DATA BEARING ON THE CROSSOVER ORIGIN OF $A^d$ FROM $A^b/A^b$ INDIVIDUALS

| Source | $A^d$ Cases | | |
|---|---|---|---|
| | Totals | Crossovers | Non-crossovers |
| $\frac{+\ A^b\ +}{lg\ A^b\ et}$ | 21 | 16 | 5 |
| $\frac{T\ A^b\ et}{N\ A^b\ +}$ | 5 | 3 | 2 |

yield of $A^d$ derivatives is so drastically reduced that, whereas approximately thirty cases had been anticipated, only five were obtained (table 3, row 2). The basis for this effect of the interchange, which turns out not to be consistent for plants of differing *A* constitution and which does not hold for nearby segments in any instance, is not yet well understood. Nevertheless, the striking reduction in $A^d$ cases from this source provides indirect evidence favoring the crossover origin of $A^d$ from $A^b/A^b$ parents since it is more reasonable to assume that the disturbance occasioned by the interchange operates to reduce a crossover, rather than a mutational, event in the $A^b$ complex. If this is the case it is expected that the crossover cases would be decreased proportionately, relative to the non-crossover types, making it more difficult, from a statistical standpoint, to correlate the occurrence of $A^d$ with crossing over. In spite of this the evidence for the latter is good since three of the five $A^d$ cases were crossovers (table 3, row 2) where less than one-fifth of the recombinant types are expected among $A^d$ cases if crossing over within the complex is not involved in their origin.

The foregoing evidence indicating that the occurrence of the $A^d$ type is associated with crossing over in $A^b/A^b$ plants, which by the alternate notation are $\alpha\beta/\alpha\beta$ in constitution, requires that the members of the $A^b$ complex undergo oblique synapsis since it would be impossible for a crossover to isolate $\alpha$ if only like components regularly synapse with each other. If $\alpha$ and $\beta$ are members of a duplication, or lie in segments constituting a duplication, the kind of pairing pattern which permits the isolation of $A^d$, the $\alpha$ component, by crossing over would be expected (figure 3). This argument requires only that the member segments of the duplication are ordered in the same direction and that they have retained synaptic equivalence.

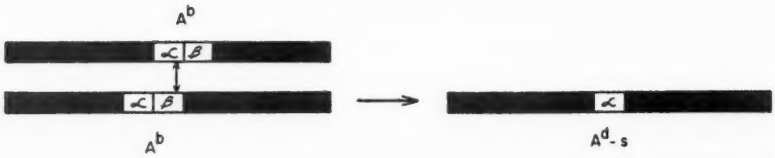


FIGURE 3. Scheme to account for the origin of the stable $A^d$ derivatives from homozygous $A^b$ individuals as a consequence of crossing over within the complex following oblique synapsis.

It may be noted here that, in spite of an earlier report to the contrary (Stadler, 1951), crossing over has recently been shown to be associated with "mutation" at the *R* locus in maize (Stadler and Nuffer, 1953; Stadler and Emmerling, 1954). The separation and isolation of the $R^r$ elements (P) and (S) relating to plant and seed effects, respectively, in homozygous $R^r$:Cornell individuals, as in the case just reviewed, is explicable only in terms of crossing over within a complex and involving obliquely-synapsed strands. This suggests that these elements too are homologous and as such are probably members of a duplication.

## THE SIGNIFICANCE AND CONSEQUENCES OF DUPLICATION AT THE *A* LOCUS

We return now to the question of order within the members of the duplication. The experiments dealing with homozygous $A^b$ ($\alpha\beta/\alpha\beta$) plants are crucial in this regard. If the duplication is of the reverse type (having the order in one member the reverse of that in the other) strands resulting from crossovers within the obliquely synapsed complex are expected to have the same fate as their counterparts in plants heterozygous for an inversion; that is, they should not be included in functional gametes. In fact, in the studies of the pseudo-alleles in Drosophila, where the associated doublet structure has been taken as cytological evidence of duplication, the absence of certain exceptional types of crossover derivatives is the best evidence available suggesting that the presumed duplication is of the reverse type (Lewis, 1945). Conversely, the association of crossing over with the rare occurrence of exceptional $A^d$ types among structurally normal gametes of $A^b/A^b$ plants indicates that the $A^b$ duplication must be of the serial type.

Established tandem duplications of the serial type have not been found in natural populations of Drosophila or Sciara in spite of ample opportunity to observe them cytologically. Their absence here has been attributed to their instability (Metz, 1947). However, as is known from the studies of Bar (Sturtevant, 1925) structural changes in this type of duplication are as likely to lead to higher levels of replication as they are to result in the loss of the duplication. This being the case, their elimination from a population would appear to require not only that they be unstable but also that duplications and higher levels of replication have a disadvantage in selection. While there is reason for considering relatively large duplications to be detrimental through their effects on genomic unbalance, this argument

may hardly be credited for most duplications of slight dimension. At any rate there is no reason to expect that all such duplications are deleterious. On the other hand, because of their structural instability, it is expected that the serial duplication would be present in some, perhaps most, but not all individuals in a population.

There are reasons for believing that serial duplications of slight magnitude, as compared with those of the reverse type, may provide extraordinary means of adding to genetic diversity in an evolutionary sense. A reverse duplication is effectively stabilized against further change. In the case of the serial duplication, however, once the initial duplicating event, which may be highly infrequent, has occurred, further replication is expected at an accelerated rate, since it is dependent merely on crossing over within obliquely synapsed members and may occur within the limits of tolerance of the organism for the duplicated chromosomal material. Replicated genes are thus free to explore new mutational pathways, perhaps even new functions. Ultimately such new genes and functions may be stabilized and incorporated into a genome through a loss of homology and pairing tendency between adjacent member genes or segments. The possible evolutionary significance of such a mechanism is obvious, and it goes without saying that this aspect of the problem of gene clusters should be a particularly engaging one for future investigation.

If this mechanism is important in the evolution of genetic systems it might be expected that serial duplications of this type would be found rather frequently. While they have not been associated with the so-called pseudoalleles in Drosophila it is interesting to note that only two loci in maize, *R* and *A*, have so far received a critical test and in both cases serial duplications appear to be involved. Moreover, it is probable that the $R^r$:Cornell and the $A^b$ alleles are not isolated instances of duplication at their respective loci. $R^r$ alleles are widely distributed in the Western Hemisphere. It is not known with certainty, but may be expected confidently, that other $R^r$ alleles, especially those for which independently mutating (S) and (P) elements are known, will be found to have a duplication basis. Likewise, $A^b$ is not an isolated instance of this kind of duplication at the *A* locus, though it is true that so far this feature is known only for certain *A* alleles of South American origin. Alleles with purple plant and dominant brown pericarp effects have been found thus far in populations from Ecuador, Peru and Venezuela. There is convincing evidence for the existence of separable $\alpha$ and $\beta$ components in several of these alleles from Peru. The results of recent studies of one of these alleles, designated $A^b$:Lima, are summarized here because they emphasize another interesting aspect of the serial duplication as it relates to changes in gene systems.

The $A^b$:Lima allele "mutates" infrequently to a form having an intermediate or pale phenotype which is weaker than that of $A^d$, the crossover derivative of the original $A^b$ from Ecuador. In fact, the pale-acting derivative from $A^b$:Lima is similar in effect to $a^p$, which itself was extracted from a Peruvian population. Recent experiments employing $A^b$:Lima/*a*

heterozygotes have established that this pale form arises from $A^b$:Lima as a consequence of crossing over, so that it may be concluded that $A^b$:Lima, like $A^b$, has separable $\alpha$ and $\beta$ components. However, the fact that the pale derivatives here are weaker in effect than $A^d$, would suggest that the $\alpha$ components in these two complexes of differing geographic origin are not identical. There is another more striking difference between the two complexes; the order of components in $A^b$:Lima, using the $C$ notation for the centromere, is $C\beta\alpha$, whereas, in the case of the original $A^b$, it will be remembered, the order is $C\alpha\beta$.

Does this mean that the $\alpha$-$\beta$ complex of $A^b$, as a whole, is inverted with respect to that of $A^b$:Lima? Assuredly not, since in heterozygotes with the same recessive *a*, both complexes yield, as a consequence of crossing over, functional gametes carrying the $\alpha$ derivatives. The crucial experiment involving a test of $A^b/A^b$:Lima individuals has been performed; from heterozygotes of this type, $\alpha$ derivatives are obtained and their occurrence is associated with crossing over. Moreover, the genetic constitution of these derivatives, with respect to marker genes present in the tested heterozygote, confirms the changed order of the components in the two complexes. The evidence indicates, therefore, that the one complex is not simply an inversion of the other but rather that the members of the duplication have exchanged position while retaining the serial order of the duplication as a whole. Such a change in sequence of members is explained, indeed is expected, only if the duplication is of the serial type and if adjacent members have synaptic equivalence. It appears therefore that changes in sequence, as well as in numbers of members, are expected in the cases of serial duplications such as $A^b$, and that such variations may prove to be characteristic of different races in nature.

## SUMMARY

Analyses of crossover derivatives from certain heterozygotes carrying the $A^b$ "allele" indicate that the separable elements of $A^b$ reside in adjacent members of a duplication. Thus the occurrence, in association with crossing over, of more than one type of derivative from each of two such heterozygotes is taken as evidence of variable local synaptic patterns reflecting homology between the $A^b$ members. Likewise, the isolation of the alpha or proximal component from homozygous $A^b$ individuals in association with crossing over is explained on the basis of exchanges within obliquely synapsed members. This interpretation requires that the $A^b$ complex is an adjacent, serial duplication. There is evidence for structural and functional variability among $A^b$ complexes of different geographic origin.

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# LINKED GENES, PSEUDOALLELES AND BLOOD GROUPS

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We are gathered here today to discuss the theory of the gene from the standpoint of the questions raised in interpreting the phenomenon designated *position pseudoallelism*. Basically, the questions would seem to concern the limits of those active patches of chromosome called loci and the role that loci play in the integration of the chromosome.

Through the years and fortified with the knowledge that chromosomes are made up of more or less contiguous active patches, each having a different effect on phenotype and each separable from the others by crossing over, we have come to regard these patches as immutable from the effects of crossing over. In doing so, we have automatically defined the locus and the gene as a non-crossover region of chromosome. During these same years we have accumulated a large body of knowledge to the effect that these same patches which are supposedly never subject to internal rearrangement by crossing over are subject to internal rearrangement, as it were, by other forces (ionizing radiation, UV light, mutagenic agents, etc.). If we now admit that crossing over can take place within the locus and, when it does, that it might occasionally be accompanied by effects which are indistinguishable from some of the mutations caused by other forces, we might then be in a better position to discuss the problems before us. In this connection it may be well to distinguish between those examples of position pseudoallelism associated with cytological evidence for twinning (doublets and the like) and those in which suggestive cytological evidence for separate loci has not been forthcoming.

We should keep in mind throughout our discussion that the mutants designated pseudoalleles interact as alleles and in this sense are still alleles and, whatever the nature of the phenomenon of position pseudoallelism, it seems clear from the action of the mutants that we are not dealing with *regular* linked genes. The latter are distinguished by the fact that they do not act as alleles and for this reason alone it might be safe to say that they never were alleles. (In general, there seems to be no reason to believe that non-allelic genes on the same chromosome are more related in descent and function than are genes on non-homologous chromosomes). Pseudoallelism and regular linkage do have one thing in common, namely, they are both associated with crossing over.

Aside from the more basic problems posed by the phenomenon of position pseudoallelism is the impact the term pseudoalleles has had on the theory of multiple alleles. Multiple alleles receive attention simply because it is

impossible to discover or *produce* recognizable examples of pseudoallelism at loci in which only one mutant is known.

Multiple allelic systems which are particularly prone to the connotation of the term pseudoalleles are those in which there is so-called physiological mosaic dominance, that is, the heterozygotes manifest phenotypes that are qualitatively equivalent to the total effect of both homozygotes (cf. Crow, 1952; Irwin, 1952). Notable among such systems are the blood groups of man, cattle and chickens. This then brings us to the subject of linked genes, pseudoalleles and blood groups.

## THE BLOOD GROUPS: GENERAL CONSIDERATIONS

"Any discussion of the application of immunological methods to genetic studies must be incomplete if it is limited, as previous reviews have been, merely to the provision by immunological methods of data about phenotypic structure not recognizable by other means" (Burnet and Fenner, 1948).

It is significant to note at the outset that all the numerous suggestions and the few explanations which have so far been advanced to the effect that linked genes (or pseudoalleles as used synonymously by certain writers) are involved in the control of blood groups do not trace to any real genetic evidence. Rather, most of them trace both directly and indirectly to the long disproved idea that there is a strict one-to-one correspondence between antigen and antibody. If the basic premise is wrong, it follows that the explanation is very likely to be wrong.

One of the few well-established facts about antibodies is that they are not absolute in their specificities. Landsteiner devoted a large part of his classical monograph (1945) to this subject. It is well, however, to keep pointing out in our discussions about antigens, antibodies and genes that the specificity of any of the antibody reagents used in blood-typing is one of degree and only relative to the limiting circumstances of our particular investigations. Never can it be said with absolute certainty that an antibody reagent *identifies a particular antigen*; yet, this idea has been fostered by many of us in the field of immunogenetics (see, for example, Stormont and Cumley, 1943). We should always be somewhat hesitant about going immediately beyond a blood-type formula (as, say, Fisher's "CDe") to presumably equivalent genes *C*, *D* and *e* when indeed all the reactions, as well as many more, symbolized in such a formula can be readily accommodated by a single antigen such as Wiener's Rh agglutinogen "$Rh_1$." We can rightfully question the use of a formula such as $C^w deF$ (see Sanger *et al.*, 1953) when it embodies serological reactions for two hypothetical antigens "d" and "F" for which no tests have been made and conveniently omits blood factor C for which tests have been made. But, such is serology when it attempts to meet a preconceived plan that was initially based on three closely linked pairs of genes (Fisher, 1947).

Serological criteria for allelism: In spite of the fact that antibodies are not absolute in their specificities we should have little difficulty in differentiating the effects of genes that are alleles from those of genes that are

not alleles. Knowing that the products of allelic genes are similar, sometimes so much alike that they cannot be differentiated except by these very special serological tests, we should expect on immunochemical grounds that they would exhibit overlapping serological reactions of varying degrees of intensity and that these varying degrees of intensity would be reflected in our serological reagents, as clearly evidenced in the cattle studies (Stormont, 1950). Then too we have the extra sensitive antibodies known as "dosage reagents" and these seem to tell us when there has been competition between alleles in the production of their end-products as indicated by reduced degrees of agglutination or hemolysis in the cells of heterozygotes. We cannot be accused of having to rely solely on the so-called conventional test of allelism, namely, gene-segregation. On the contrary, we can cite the blood-group systems B, C, F-V and Z of cattle (Stormont, *et al.*, 1951; Stormont, 1952) all of which were first recognized on the basis of serological criteria for allelism rather than on tests for segregation. It is significant, however, that the serological and genetical criteria for allelism have never disagreed.

The synthesis of serological complexity: Many of us have no doubt wondered why the products of most of the alleles in multiple allelic blood-group systems appear to be so serologically complex while those in two-allele systems are never represented by more than one blood factor per allele. The main reason for the simplicity in two-allele systems is that we have no way of exposing cross or overlapping reactions when the studies are confined to the one species under investigation. Consequently, in two-allele systems an *apparent* one-to-one relationship between antigen and antibody always obtains. We must have at least three alleles in order to expose cross reactions. For example, let us synthesize a breed of cattle possessing only the two B alleles $B^{BGKO_2Y_1A'E'_3K'4,6,8}$ and $B^{O_1T_1E'_3I'K'}$ which we shall designate by their respective codes $B^{28}$ and $B^{56}$ (see table 4). In isoimmunization involving two-allele systems, only the two homozygotes can produce antibodies. In our example, the $B^{28}$ homozygote would produce antibodies against antigen B56 in or on the cells of the $B^{56}$ homozygote or the $B^{28}/B^{56}$ heterozygote. None of the antibodies produced against B28 in a $B^{56}$ homozygote would react with antigen B56 and none of the antibodies produced against B56 in a $B^{28}$ homozygote would react with antigen B28. There is then, in principle, no antigen other than the respective homologous antigens B56 and B28 with which to test our anti-B56 and anti-B28 sera. Consequently, these antisera would behave as mono-specific blood-typing reagents no matter how complex they might be. Accordingly, each of the antigens would be represented by a single blood factor and the complexity seen in their original formulation completely disappears.

Cross reactions become exposed whenever a third allele is introduced into a two-allele system. For example, suppose in the foregoing example that $B^{56}$ mutates to $B^{O_1T_1E'_3K'}$, coded $B^{54}$, and becomes established. Fractionation of most of the reagents produced against B56 on absorbing with the blood of $B^{54}$ homozygotes or $B^{28}B^{54}$ heterozygotes might then be

expected since B54 does not have the I′ factor shown in the formula of B56. On the other hand, antisera produced in $B^{28}$ homozygotes against B54 would be expected to react with and be absorbed by B56 since the formulation (table 4) shows that B56 has all the blood factors of B54. Consequently, the relationship between B56 and B54 would be that of serological subtypes analogous with the difference between $A_1$ and $A_2$ of man, or $T_1$ and $T_2$ of cattle, etc. We would in effect have an $A_1$-$A_2$-B system where B56 simulates $A_1$, B54 simulates $A_2$ and B28 simulates B.

As the number of alleles increases, the greater becomes the number of cross or overlapping reactions exposed among their products so that it becomes virtually impossible to develop antibodies which react with the products of individual alleles to the exclusion of all others in the set. It follows, therefore, that the products of an extensive set of alleles affecting blood groups can be differentiated only by the distinctive patterns of their overlapping reactions. Hence, the serological complexity of such multiple allelic systems as Rh of man (see Wiener *et al.*, 1952), A and B of chickens (Briles *et al.*, 1950) and the B and C systems of cattle (*loc. cit.*) is readily resolved. Complexity in serological pattern is always relative to the number of alleles (*cf.* Cotterman, 1953).

## COMPATIBILITIES AND INCOMPATIBILITIES AMONG BLOOD FACTORS OF A SYSTEM

As pointed out by Wright (1953), in such systems as B of cattle, there is no approach to the randomness of combination expected among blood factors if there were multiple loci capable of exchange by crossing over, even at rates comparable to those of mutation in rarity. There is instead the appearance of complex pattern in which the blood factors exhibit all grades of dependence up to complete dependence of one on one or more others, and there are also all grades of incompatibility up to the complete incompatibility which has suggested allelism in the narrow sense.

In the cattle studies already referred to there are numerous examples of the apparent complete dependence of one blood factor on one or more others. Most of these dependencies are embodied in the subtype symbolism of single blood factors such as O (subtypes $O_1$, $O_2$ and $O_3$), T ($T_1$ and $T_2$), Y ($Y_1$ and $Y_2$), etc. The subtype $O_1$, for example, embodies two serological properties shared by $O_2$ and one shared by $O_3$. The steps are linear, going from greatest serological complexity, $O_1$ with blood factors 1, 2 and 3, $O_2$ with blood factors 2 and 3, to least complexity $O_3$ with blood factor 3 alone. Here, factor 1 appears to be dependent on both 2 and 3, and 2 appears to be dependent on 3.

The transition from linear series of associated blood factors to nonlinear series is perhaps best depicted by the blood factors B, G and K of the B system of cattle which to date have been recognized in only five combinations BGK, BG, B, G and the absence of all three factors. Blood factor K appears to be completely dependent on both B and G, but B and G, occurring independently of K, exhibit a high degree of incompatibility.

TABLE 1

HERITABLE COMBINATIONS OF THE BOVINE BLOOD FACTORS B, G AND K AND OF THE HUMAN BLOOD FACTORS c, e AND f

| "BGK" combinations | Incidence of BGK combinations | "cef" combinations | Incidence of cef combinations |
|---|---|---|---|
| BGK | Very common in Guernseys and Jerseys; less common in Holsteins, Swiss and Shorthorns; not found in Herefords. | cef | Very common in Negroids; common in Caucasians and uncommon in Mongoloids. |
| BG | Rare in Holsteins and Guernseys; rare or absent in other breeds studied. | ce | Only one example to date. |
| B | Very common in some breeds to relatively uncommon in others; not found in Herefords. | c | Fairly common in all races. |
| G | Very common in Holsteins; relatively uncommon to absent in other breeds studied. | e | Very common in Mongoloids; common in Caucasians and Negroids. |
| -* | 100 per cent of Herefords studied; very common to common in other breeds. | -* | Rare in Caucasians; not recognized to date in other races. |

* Dash (-) indicates absence of all three blood factors.

It is of interest to mention the BGK relationship here because of a very similar relationship which seems to be developing in regard to the Rh blood factors c, e and f. So far, c, e, and f have been observed only in the combinations cef, ce, c, e and the absence of all three factors (from data of Sanger *et al.*, 1953). A comparison of the relative frequencies of the heritable "BGK" and "cef" combinations is given in table 1. A considerable degree of likeness between BGK and cef is indicated. If the dependence of f on both c and e turns out to be anywhere near the intensity of that of K on both B and G (Stormont, 1950), it will be difficult to insist that more than one gene is involved in the control of these three blood factors (cf. Sanger *et al.*, 1953).

In table 2 are summarized the incompatibilities of each of 24 blood factors in the B system (including new factors designated 4, 6, 7 and 8) currently being studied in our laboratory. Each of these 24 factors shown in the left column of table 2 is oppositional to or incompatible with at least two or more of the others. Factor B is oppositional to $E_1'$ and $J'$. Glancing down the column we note that $E_1'$ is oppositional to $J'$ which suggests what at first sight would appear to be a set of allelic blood factors "B-$E_1'$-$J'$" and thereby a set of allelic genes *B*-$E_1'$-$J'$. Similarly, we note that G-P-$T_1$-$J'$ forms a second set. But, on the assumption of a separate locus, we

TABLE 2

OPPOSITIONAL RELATIONSHIPS OF 24 BLOOD FACTORS IN THE B SYSTEM OF CATTLE

| Blood factors | Oppositional to the blood factors |
|---|---|
| B | $E_1'$ and $J'$ |
| G | P, $T_1$ and $J'$ |
| I | K, P, $T_1$, $Y_2$, $A'$, $D'$, $E_2'$, $E_3'$, $I'$, $J'$ and $K'$ |
| K | I, $O_1$, P, Q, $T_1$, $T_2$, $D'$, $E_1'$ and $J'$ |
| $O_1$ | K, P, $E_1'$, $E_2'$ and $J'$ |
| $O_x$ | P and $E_1'$ |
| P | G, I, K, $O_1$, $O_x$, $T_1$, $T_2$, $Y_1$, $A'$, $D'$, $E_1'$, $E_2'$, $E_3'$, $J'$ and $K'$ |
| Q | K, $T_2$, $Y_1$, $Y_2$, $A'$, $E_2'$, $E_3'$, $J'$ and $K'$ |
| $T_1$ | G, I, K, P, $A'$, $D'$, $E_1'$, $E_2'$ and $J'$ |
| $T_2$ | K, P, Q, $Y_1$, $Y_2$, $D'$, $E_1'$, $E_2'$, $E_3'$, $I'$, $J'$ and $K'$ |
| $Y_1$ | P, Q, $T_2$, $E_1'$ and $E_2'$ |
| $Y_2$ | I, Q, $T_2$, $E_2'$, $E_3'$ and $K'$ |
| $A'$ | I, P, Q, $T_1$, $E_1'$, $E_2'$ and $J'$ |
| $D'$ | I, K, P, $T_1$, $T_2$ and $E_2'$ |
| $E_1'$ | B, K, P, $O_1$, $O_x$, $T_1$, $T_2$, $Y_1$, $A'$, $J'$ and $K'$ |
| $E_2'$ | I, $O_1$, P, Q, $T_1$, $T_2$, $Y_1$, $Y_2$, $A'$, $D'$, $I'$, $J'$ and $K'$ |
| $E_3'$ | I, P, Q, $T_2$, $Y_2$ and $J'$ |
| $I'$ | I, $T_2$, $E_2'$ and $J'$ |
| $J'$ | B, G, I, K, $O_1$, P, Q, $T_1$, $T_2$, $A'$, $E_1'$, $E_2'$, $E_3'$ and $I'$ |
| $K'$ | I, P, Q, $T_2$, $Y_2$, $E_1'$ and $E_2'$ |
| 4 | I, P, Q, $T_1$, $T_2$, $Y_2$, $D'$, $E_1'$, $E_2'$ and $J'$ |
| 6 | I, P, Q, $T_2$, $Y_2$, $D'$, $E_1'$, $E_2'$ and $J'$ |
| 7 | P, Q, $T_1$, $T_2$ and $E_1'$ |
| 8 | $O_1$, P, Q, $T_1$, $T_2$ and $E_1'$ |

are already in difficulty since $J'$ would appear to be represented by genes at each of the implied loci. As we proceed, the difficulties increase rapidly; $J'$ appears in 22 different sets; I appears in eight different sets; K appears in six different sets; etc. In fact there are far more of these sets than there are known blood factors in the B system. Most of us would hesitate in postulating a separate locus for each of these oppositional sets. Consequently, the only interpretation based on linked genes which has so far come out of the cattle work is that summed up by Irwin (1949) when he stated that genetically these *complexes* (i.e., the inherited blocks of blood factors $BGKO_2Y_1A'E_3'K'$, $BGIO_1T_2A'$, $BO_1Y_2D'$, $GY_2E_1'$, $IQE_1'$, $O_1T_1E_3'I'K'$, etc. of the B system) may be accounted for by assuming closely linked genes, each of which affects a respective part of the antigenic complex. Carrying this proposal to its logical conclusion, it would be necessary to postulate a separate locus for each blood factor in a system—or upwards of 24 linked loci in the case of the B system of cattle. Each blood factor such as B, G, I, K, etc., would then have a single alternative b, g, i, k, etc. for which antibodies are yet to be discovered. In the absence of any supporting evidence, such an explanation has been difficult to sustain; it is altogether too much like a pyramid built on its apex.

TABLE 3

HERITABLE COMBINATIONS OF KNOWN CDEF BLOOD FACTORS

| Combinations* | Wiener's designations | Incidence (mainly after Wiener et al., 1952) in | | |
|---|---|---|---|---|
| | | Caucasians | Negroids | Mongoloids |
| cef | rh | Common | Less common | Rare |
| cDef | $Rh_o$ | Relatively uncommon | Most common | Relatively uncommon |
| ce | $rh^x$ | One example | Not yet found | Not yet found |
| cE | rh'' | Rare | Rare | Rare |
| Ce | rh' | Rare | Rare | Rare |
| CE | $rh_y$ | Very rare | Very rare | Very rare |
| $CC^wE$ | $Rh^w_y$ | One example (?) | Not yet found | Not yet found |
| $CC^we$ | $rh^{'}_{w}$ | Extremely rare | Extremely rare | Extremely rare |
| CDe | $Rh_1$ | Common | Common | Most common |
| cDE | $Rh_2$ | Relatively common | Relatively common | Relatively common |
| CDE | $Rh_z$ | Very rare | Very rare | Very rare |
| $CC^wDe$ | $Rh^w_1$ | Uncommon | Uncommon | Uncommon |
| CD | Rh | Not yet found | Rare | Not yet found |
| D | $Rh^x_o$ | Very rare | Not yet found | Not yet found |

*The serological voids "d" and "F" are omitted. The symbols $C^u$, $D^u$ and $E^u$ substitute freely for the respective symbols C, D and E. The heritable combinations involving the blood factor $c^v$ (meaning positive with both anti-C and anti-c) are omitted for lack of information.

The heritable combinations of the known Rh blood factors C, $C^w$, $c^u$, c, D, $D^u$, E, $E^u$, e and f, as deduced from data given in Race and Sanger (1950) and by Sanger *et al.*, (1953), are listed in table 3. From table 3, the following sets of oppositional blood factors are derived: C-$C^u$-c (questioned on the basis of the meaning of $C^v$ (see footnote to table 3)), C-$C^u$-f, $C^w$-E-$E^u$-f (or possibly $C^w$-f and E-$E^u$-f, if the combination $CC^wE$ (table 3) becomes definitely established), $C^w$-$C^u$-f, $C^w$-$C^u$-c, D-$D^u$ and E-$E^u$-e. The most obvious feature of these sets is that they bear little relationship to the conventional CDEF sets C-$C^w$-$C^u$-c-$c^v$, D-$D^u$-d, E-$E^u$-e and F-f. The sets shown here, however, are not based on hypothetical linked antibodies (as, for example, linked C-$C^w$ presumed to be present in all standard anti-C reagents), or on numerous predicted chromosomes (as, for example, CDef, cDEf, cDeF, etc.) yet to be discovered, or on predicted antibodies yet to be discovered. In viewing the conventional sets D-$D^u$-d and F-f we should keep in mind that the symbols "d" and "F" are purely serological voids.

Perhaps the most noticeable feature about the CDEF explanation (Sanger *et al.*, 1953) as compared with the original CDE explanation (Fisher, 1947) is that there is no longer correspondence between sets of oppositional blood factors and the proposed sets of linked genes. The reason for this seems clear when we note, for example, that blood factor f appears in at least three different sets. As more blood factors are added to the Rh system, the situation regarding both compatibilities and incompatibilities has become very comparable on a lesser scale to that seen in the B system of cattle. As in the B system, the most conservative approach from the stand-

point of linked genes would appear to be that of calling forth a new locus each time a new blood factor is added to a system (cf. Rosenfield *et al.*, 1953, and Sanger *et al.*, 1953). There is, of course, as in the B system, a limit to the number of linked loci that may be imposed even if we assume absolutely linked genes, a possibility mentioned by Fisher (1947). The reason for this becomes clear when we consider the origin of heritable combinations of blood factors within a system.

Assuming closely but not absolutely linked genes, both crossing over and mutation would be operative in producing new combinations of blood factors, but on the assumption of absolute linkage only mutation would be effective. In the absence of any direct evidence for recombination, it becomes apparent, as more and more loci are called forth, that mutation is the only real force in producing the heritable combinations of blood factors within a system. In this connection it is interesting to call attention once again to the "BGK" and "cef" relationships given in table 1. Here, for example, mutation of *B* to *no-B* and *G* to *no-G* in a hypothetical gene-complex *BGK* would be expected, in the course of evolution, to produce numerous examples of the combinations BK and GK. Similarly, we should expect numerous examples of the combinations cf and ef. The fact that such combinations have not been found suggests that the hypothetical linked genes do not exist or that they are not independent in mutation. In the latter event it would be difficult to escape the conclusion that they are embodied in a single gene, the B gene in one case and the Rh gene in the other.

## IMMUNOCHEMICAL CONSIDERATIONS

If the complex blood-group systems are controlled by multiple alleles, we can expect that the immunochemical approach will eventually provide evidence to the effect that the blood factors within such systems are purely the serological integrants of antigens (or haptens) of a homologous series rather than being separable antigens in themselves (after Stormont *et al.*, 1951, and Wiener and Wexler, 1952). In this connection, it is interesting to point out that Race and Sanger (1951, p. 128) mentioned work underway on Rh in their unit which "has led to the conception of a basic raw material which can well be identified with the carrier" (interpreted here as "hapten"). Possibly, then, there is already evidence to the effect that the so-called "elementary antigens" C, D, E, etc. do not exist as immunochemically separable entities, *a consequence predicted by multiple alleles.*

Those pathways of serological cross reaction traced by means of the sets of oppositionally related blood factors already referred to can be interpreted as relating mainly to differences in isomerism or surface configuration in antigens of a homologous series. Asymmetry, especially stereoisomerism, is known to have a marked effect on serologic and enzymatic specificity, differences in stereoisomers being readily detected by antibodies (see Landsteiner, 1945) and sometimes by appropriate enzymes. In this connection, it is interesting to point out that the sharpest differ-

ences within a blood-group system, as B of cattle, are those involving incompatible blood factors. Various substituent groups (say, COOH, OH and $CH_3$) at a number of intramolecular antigenic sites might then account for compatibilities. Steric hindrance, that is, the blocking effect due to the strategic placement of relatively inert substituent groups or the disadvantageous placement of active groups, might play the predominant role in eliminating whole blocks of serological reactions (see Pressman, 1953). Consequently, it is hardly necessary to conceive that the absence of certain reactions is invariably due to the absence of gene-product and thereby the gene, as implied by the symbol "-D-" of CDE (see Race and Sanger, 1951).

If the blood-group antigens or haptens of certain systems are polysaccharides and the controlling alleles are nucleoproteins, it is not expected that antibodies directed against gene-products would cross react with the genes. Consequently, it is difficult if not impossible to carry over cross reactions seen at the level of the antigen to structure at the level of the gene. Such would be the case if there were a one-to-one-to-one correspondence between antibody, blood factor and gene but such a correspondence does not exist. Therefore, in immunogenetic studies, as in other genetic studies, the most immediate approach to an understanding of the gene is through the study of the effects of gene mutations, particularly spontaneous mutations.

## MUTATIONAL EFFECTS

From the standpoint of studies of the effects of gene mutation, the B system of cattle is ideal not only because of the large number of alleles (100 now recognized in our laboratory) but also because of the ease with which mutational effects may be identified. For example, almost any mutation in a B allele having effects on serological specificity, no matter how minute the change might be, should be detected by the very sensitive battery of B reagents now in use.

In table 4 are listed 30 representative *phenogroups* (the products of individual alleles) in the B system of cattle. These 30 phenogroups, each followed by a code-designation for convenient reference, are arranged in nine sets, the BGK set, the I set, the T set, etc. Although there are numerous possible arrangements of B phenogroups, that given in table 4 suffices in illustrating the marked between-set differences as contrasted with the general similarity of phenogroups within sets.

It is the similarity of phenogroups within sets as contrasted with the dissimilarity between sets that has suggested two possible orders of mutational effects. The first of these is concerned with effects which amount to the replacement or elimination of single blood factors and changes in subtypes of single blood factors. For example, as we compare the phenogroups coded B27 and B28 of the BGK set (table 4) and B53, B54 and B56 of the T set we note that the within-set differences in this example largely involve blood factors I′ and K′, as if I′ could be substituted for K′ and

TABLE 4

REPRESENTATIVE HERITABLE COMBINATIONS (PHENOGROUPS) OF BLOOD FACTORS IN THE B SYSTEM OF CATTLE ARRANGED IN 9 SETS

| BGK set | | I set | | T set | |
|---|---|---|---|---|---|
| | *Code* | | *Code* | | *Code* |
| $BGKE'_2 7,8$ | B12* | BI | B22 | $O_1T_1E'_3I'$ | B53 |
| $BGKO_3A'K'6,8$ | B19 | GI | B30 | $O_1T_1E'_3K'$ | B54 |
| $BGKO_2Y_1A'E'_3I'6,7,8$ | B27 | $IE'_1$ | B43 | $O_1T_1E'_3I'K'$ | B56 |
| $BGKO_2Y_1A'E'_3K'4,6,8$ | B28 | $IQE'_1$ | B44 | $T_1E'_3$ | B74 |
| **$O_1$ set** | | **P and Q set** | | **$E'_1$ set** | |
| $BO_1$ | B3 | P | B65 | $GY_2E'_1$ | B39 |
| $BO_16$ | B5 | PI' | B68 | $QD'E'_1$ | B71 |
| $BO_1Y_2D'$ | B16 | PQI' | B69 | $E'_1$ | B85 |
| **D' set** | | **J' set** | | **Residual set** | |
| GD' | B33 | $O_3J'7,8$ | B60 | -** | (b) |
| $O_xY_1D'$ | B77 | $O_3J'K'7,8$ | B62 | $BO_2Y_1A'E'_3$ | B22 |
| $O_xD'E'_38$ | B84 | $O_3Y_1D'J'K'7,8$ | B63 | $O_xE'_38$ | B90 |

*Code-designations now in use by several laboratories.

**Dash (-) indicates no reaction with any of the known B system reagents.

the converse. Two examples bearing on this suggested order of mutational effects have already been obtained (Stormont, 1953). Briefly, one of the suggested changes was from B5 to B3 in the $O_1$ set. The other involved phenogroup B39, ($E'_1$ set of table 4) which was transmitted in a modified form, namely "$GY_2E'_3$." Here the mutational effect involved a difference in the subtypes of the blood factor E'. The possibility that either or both of these anomalies in the appearance of B phenogroups is consequent on epistasis rather than gene mutation cannot be ruled out at this time.

The second order of mutational effect suggested by data in table 4 is that involving transitions of whole blocks of blood factors as, for example, "BGK8" and "$O_1T_1E'_3$." In this way the change involved in going from a phenogroup in one set, as BGK, to another set, as T, might be accomplished in a single step. Although there is as yet no direct evidence for this proposed order of change, we recall from the foregoing discussion that "BGK" probably changes to "no-BGK" in a single step. No-BGK could be any of a number of serological effects as B or G or I or even $O_1T_1E'_3$.

Finally, we need to consider the possible effects of gross rearrangements in genic material associated with crossing over, namely, the pseudoallelic effect. Here again, there is as yet no direct evidence bearing on the problem. On the assumption that a blood-group locus may occasionally be affected by crossing over and that at least a few of the rearranged alleles might survive, it seems possible that the phenogroups produced by such alleles in complex systems such as B of cattle would bear little resemblance to those produced by the parental alleles involved in the rearrangements. One thing seems certain, that there would be no position ef-

fects and the difficult concept "wild type" would not enter into the interpretation of the results. Consequently, it would be impossible to partition the rearranged allele into hypothetical *a* and + or + and *b*, or *a* and *b* or + and + components. The rearranged allele would behave as a single functional unit. Likewise, the thought occurs that conventional pseudoallelic symbols as *a*+, +*b*, *ab* and ++, which imply two loci separable by crossing over, can each be regarded as single physiological units, the alleles of a single gene (cf. Goldschmidt, 1954, and Pontecorvo, 1953). In this connection it is interesting to note that the alleged independent action of a pseudoallelic element such as *a* in a chromosome *a*+ can no more be separated from + than + can be physiologically separated from + in the chromosome ++.

## SUMMARY

The theory of closely or absolutely linked genes and of "pseudoallelic" genic elements as applied to blood groups is critically examined both at the level of the antigen and at the level of the gene. The most complex system of blood groups recognized to date in any species, namely the "B" system of cattle, is used as a model throughout the discussion. Comparisons are drawn between certain features of the B system and the Rh system of man in regard to the kind of explanation involved in the "CDE" symbolism of the Rh blood groups. At the outset it is pointed out that the notion of linked genes and/or pseudoalleles traces to the erroneous impression that a single specificity is the definitive characteristic of an antigen. Hence, the theory of linked genes has constituted a problem in the study of blood groups only insofar as it has led to confusion in interpreting serological results. The hypothesis of multiple alleles proposed to explain the more complex systems of blood groups still exist and can be maintained even though the time may come when "crossover" mutants are recognized and differentiated from mutations in these alleles caused by other forces.

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# SUMMARY, SYNTHESIS AND CRITIQUE*

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Unlike the other contributors to this Symposium I have no array of data to present in favor of this or that interpretation of pseudoallelism and its bearing on gene theory. I have been told that it is my job to "synthesize the syntheses," but all I agreed to do was to try to see "as through a glass darkly" and with as little personal astigmatism as possible.

I have felt for some time that much of the controversy during the past few years on the nature of the gene turns as much on differences in detail as on basic differences in ideas. If one could see the wood instead of the individual trees, I feel that a respectable common denominator of agreement would be found. A search for the common denominator at this time is premature and must be somewhat superficial, but there are certain advantages in making an attempt. It seems to me that though as people trained in the scientific method we rightly prize precision in act and statement, yet we are in danger of following the legal people into that morass where no simple idea can be conveyed without a page or two of instructions, definitions and exceptions. I hope we are not forcing ourselves to follow them to the point of no return. If you do not see cause for anxiety let me point out that at a meeting like this, a genetics meeting, it is difficult to talk about "The Gene." Is it Stormont's "single functional unit," one of Laughnan's "separable components," Lewis' paired entities "which exhibit *cis* and *trans* effects" or Green's physical cum physiological "unit"?

In recent years there has been much marshalling of defences to meet Goldschmidt's real or fancied attack on the particulate nature of the gene. Since the speakers at this Symposium are all apparently "pro-particulate" we can accept this as a provisional starting point, and, for the time being, relapse to cold war status with the "anti-particulate" forces. Three of the speakers, Lewis, Laughnan and Green, have presented pictures of situations which have more features in common than they have discrepancies. Each has studied in detail one or more "families" of mutant forms. Each family of mutants traces back to changes involving a relatively small segment of a chromosome: the members of each family closely resemble one another in their mutant properties. Critical studies show that the members are not simple alternatives as would be the case if they represented different but mutually exclusive changes at the same physical site. Instead they are shown to result from changes at two or more nearly located, though

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not necessarily contiguous sites. As a consequence they have the same physical potentialities of existing separately or in combination as does any set of closely linked genes. In the Drosophila cases studied there is independent evidence that the serial arrangement is not a matter of chance but is associated with a physical duplication of sites. In Laughnan's case there is at any rate no reason for rejecting a similar mode of origin. Indeed the oblique pairing which involves *either* the alpha *or* beta component in his material would be the expected consequence of a tandem repeat without inversion. But perhaps the strongest evidence in favor of duplication as a common mode of origin comes from Green's Beadex loci (to which reference has not been made this afternoon). The hypercritical might object that the association of an *apparent* physical duplication of a band to produce a doublet, with the *apparent* duplication of mutant properties might be a matter of chance. The fact that a duplicate *sequence* of loci, including Beadex, exhibits similar phenomena to that characteristic of presumed simple repeats places the role of duplication in the origin of pseudoalleles on a rather firm foundation. Here there can be no doubt concerning the physical duplication.

One of the aspects of pseudoallelism which has been particularly "woolly" in the past seems to be clarifying. This is the *cis-trans* effect which at one time seemed characteristic of pseudoalleles in Drosophila and absent from other material. Lewis' finding that the same pseudoalleles may or may not show *cis-trans* differences according to other structural conditions in their neighborhood shows that we are dealing with differences in detail on a common basic pattern, rather than basic differences between organisms. This means probably that we can cut out the semantic gymnastics in which we have indulged in trying to differentiate between the two. But it does not explain why the position effect associated with *cis-trans* relationships should be common in Drosophila and rarely met with elsewhere.

One of the interesting consequences which seem to be emerging from these studies is that the more rigidly one applies the particulate concept in making interpretations of pseudoallelic effects, the less important become the properties of the gene *per se* and the more important its spatial relationships with its neighbors. One is forced to move away, I think, from the concept of autonomous units each with their own unique properties towards the possibility of a system where all the units may be potentially similar but where unique properties may or may not develop according to the spatial relationships of the position they happen to occupy. One starts with a single particulate unit, or gene, occupying a specific position in a chemical chain—the chromosome. In this position it has specific properties and specific mutant forms. It then becomes duplicated and as a consequence neither of the daughter units can occupy exactly the same position as the parent unit, and the new bonding between the daughter units must modify spatial relationships. One would not therefore expect the daughter units to have exactly the same properties; if they did pseudoallelism would probably

not have been discovered. And if spatial arrangements were not important we should not expect position effects.

Now if pseudoallelism is the end point of a blind alley there is no more to be said. If on the other hand it is merely the beginning of a sequence of changes, it is clear that there would be the possibility of continuing divergence in properties with each successive duplication or other structural rearrangement. It is surprising that Goldschmidt has condemned this suggested process as "mystical" because if one develops a theory along these lines one reaches a point of view not far from his own, namely, that the properties of a chromosome or chromosome segment are determined by the pattern of the whole rather than the sum of its separate components. Since one has to have parts to make a pattern, I see no difficulty in continuing to call the parts, genes. Furthermore, other examples of this mystical process are to be found which are factual, not hypothetical. It is a reasonable supposition that all individual carbon atoms are essentially similar, yet when they are linked together in a fatty acid they develop unique potentialities as is shown by the isomerism which results when the same halogen atom or other substituent group is introduced in alternative positions in the chain. Or, at a much higher level of organization, it is clear that the specific properties of a particular protein or nucleic acid are not explicable in terms of the additive effects of their rather simple components. The higher the level of organization, it would seem, the less important become the properties of the building material and the more important the manner in which it is arranged, for the determination of its specific properties. Is it reasonable to avoid these principles in developing the theory of the gene?

To return to the origin of pseudoalleles: Lewis has pointed out elsewhere that a change in properties accompanying duplication would imply a changed gene-substrate relationship. Assuming that the change in properties was very slight, two plausible hypotheses were developed: (1) that the daughter genes would use the same substrate to synthesize different products (competitive system), (2) one daughter gene would utilize the primary product of the other (sequential system). These are only two out of several possible models, but the second has achieved some success in providing a formal explanation of the *cis-trans* effect where the former is associated with wild type, and the latter with mutant expression. It has another attractive feature, too, in that a sequential system would represent a step in organization, the daughter genes performing interdependent steps in a common synthetic pathway. However, it seems hardly likely that the sequential system can be generalized to fit all cases of pseudoallelism. First, it appears now that the *cis-trans* effect is a special form of a general phenomenon. Second, cases have been analyzed recently (Fox, unpublished) in which the properties of mutants in a pseudoallelic complex cannot be arranged in a simple step-wise sequence.

It is time to consider where Stormont's antigen relationships fit into the pattern, if they fit at all. McClintock's analysis of the pale yellow/yellow green interaction in corn (which is still perhaps the most clear-cut case of

psuedoallelism known) provided a model which it was tempting to apply to all provocative allelic series which did not form regular sequences. The blood groups in particular appeared to be "sitting ducks" for pseudoallelic snipers. It now appears that we may have been trigger happy. In our enthusiasm we may have forgotten that concepts of spatial organization as related to specificity were not developed from gene theory but were borrowed from the immunologist and enzymologist. Stormont's data suggest that we may have been trying to fit two levels of organization into one. The situation is somewhat analogous to one which provides a recurring problem to taxonomists. It starts something like this: attention is directed to a specialized group of plants of uncertain taxonomic affinities. The first effort in handling them is to lump them under one name and characterize them as a "variable species." This is unsatisfactory but the situation remains pigeon-holed until some enthusiastic individual subjects it to a detailed study. He comes out with an entirely new classification in which practically every variant carries its own specific rank. Diagrammatically his classification can be represented as a long horizontal strip, divided into equal sized boxes, each box corresponding to a species. More sober reflection prevails, more information is obtained, and eventually it seems better to reduce the number of species, and to subdivide those that remain into hierarchies of sub-specific rank. The new model is therefore two-dimensional, involving two or more levels of organization.

Like the taxonomist who tries to make a new species to accommodate every new variant wehave tried to make a new pseudoallele to accommodate every new antigen. Maybe we should now consider the likelihood of more than one level of organization. I do not think that this means necessarily a complete retreat to the single gene unit comprising an almost infinite variety of mutant expressions. This would be equivalent to scrapping the taxonomist's horizontal strip in order to return to the single variable species. On the contrary it may be that a study of cross-relations between antigens at the one level of organization may provide valuable clues concerning cross-relations of pseudoalleles at the other, possibly in more precise terms than are presently available.

A few pages ago I admitted that a search for a common denominator among the ramifications of this fascinating topic would necessarily be premature and superficial at the present time. But this at least would seem fairly obvious: any new concepts we are tempted to form of the nature of the gene must be referable to a chemical model if they are to be constructive. Morphological models are no longer sufficient. Recent work on the structure of nucleic acids which was summarized by Watson at the Oak Ridge Conference this year, coupled with the knowledge of protein structure already accumulated, point to the importance of a linear pattern involving what appear to be rhythmic alternations of repeated chemical sequences. If the properties and structure of the chromosomes can be fitted into this model, then the properties and structure of genes will have to be fitted in also. At the most

elementary level we should expect that the organization of genes in the chromosome would have the general properties of the components of an organic chemical chain. In this situation it is perhaps excusable to look for analogies, formal though they be. One of the properties of a chemical chain is the capacity to form isomers, a consequence of repetitions in the sequence. We should expect to find isomers rather frequently in a genetic chain, and perhaps we have them in the form of pseudoalleles. In simple chains, isomerism is purely a linear property, that is, it is determined only by the position along the chain in which the substituent group is inserted. In other chains double bonding may impart rigidity to the structure, so that if two substituent groups are involved it makes a difference whether they are inserted on the same or opposite sides of the chain. The two geometric isomers that result are known as *cis-trans* forms, from which presumably the same nomenclature in the Drosophila position effects was originally derived. Speculations have already been made that position effects may be in some way related to somatic pairing and the consequent more precise spatial arrangement between homologues in Drosophila. One might perhaps speculate further and consider the possibility that both somatic pairing and position effects might result from a more rigid bonding in the chromosomes of this organism which might make geometric isomerism more likely to occur.

Another general property of organic chemical chains is that other things being equal, the longer the chain the greater the variety of derivatives which are obtainable. The possibility of increasing the number of genes, and hence presumably the potentialities for evolution, through duplications of the repeat type has frequently occurred to geneticists. Duplications ranging from single genes to whole chromosomes are the only known mechanisms of increasing the number of genes per individual, and indeed it is difficult to visualize any other mechanism which might be operative within the requirements of a self-duplicating chain structure. The fact that mechanisms of gene duplication are known to exist does not of course prove that they are of significance in evolution. On this point, I think, we are likely to obtain evidence more quickly from comparative studies of polyploid series than from studies of repeats. Although such studies lie outside the scope of this Symposium, I should like to mention some general principles which seem valid. The genus Gossypium provides particularly suitable material for comparative studies of diploid and amphidiploid species. In Gossypium, when one considers gene loci which are common to amphidiploid and both putative diploid species, one usually finds that the locus is represented only once in the amphidiploid. When it is represented twice, mutants at one locus tend to be confined to one amphidiploid species, and mutants at the duplicate locus are confined to another amphidiploid species. In general, then, each amphidiploid species tends to be functionally diploid. One can explain this situation by supposing that one of the originally duplicated loci has become lost or inactivated. It does however require an effort of will power to imagine how the dominant amphidiploid group of today

achieved its success by carrying around about fifty per cent of its genetic content in an inactive form. An alternative explanation is that the originally duplicate loci have changed in function. In support of this interpretation is the fact that we find some "duplicate" loci which exhibit much the same degree of differentiation as the pseudoalleles which have been described today. But better evidence may be available. Certain *recessive* mutants are known in amphidiploids which apparently have no counterpart in any diploid species. Some time ago I suggested that if these represented mutants at *new* loci, unrepresented in diploids, then they should behave as *dominants* on crossing with diploids. (This of course is the expected effect of placing a recessive against a deficiency). Several recessives so tested gave negative results, but recently Gerstel has found two mutants, both unknown outside the amphidiploid species, which give semi-dominant reactions on crossing with diploids. I think it is quite likely that in Gossypium we shall be able to find a continuous series of loci which collectively bridge the gap between strict duplication and complete independence in function.

I have mentioned these indications briefly because they are in line with the theory that duplication may lead to the origin of new loci; this is now somewhat more than an attractive speculation. It follows that a better understanding of pseudoallelism may have a very wide significance apart from its obvious importance for interpreting the nature of the gene.

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# SPERM UTILIZATION AND BROOD PATTERNS IN DROSOPHILA MELANOGASTER

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The question of brood patterns as a manifestation of differential sensitivity of sperm irradiated at various stages of spermatogenesis has recently been attracting considerable attention. The same question is involved in work with chemical mutagens. The usual experimental procedure for obtaining successive broods from males has been to mate them to new females, either in pair or mass matings, at intervals of 3 or 4 days. There is no direct experimental evidence that this system of mating affords maximum sperm utilization. Lüning (1952, 1954) claims that when males are not allowed to mate the sperm are resorbed and/or ejaculated without copulation. Baker and von Halle (1953), on the contrary, base their arguments for the recovery of chromosome breaks on the assumption that sperm are stored. Auerbach (1954) has been keenly aware of the problem and by using a more sensitive mating system, 1 ♂ × 3 ♀♀ for 3 day periods and a very refined technique, has obtained sharper distinctions between the stages of spermatogenesis.

It should be possible to avoid uncertainties of this nature by devising a mating technique which would ensure maximum utilization of all mature sperm in order to avoid overlapping or displacement in time of inseminated sperm treated at various stages. The experiments reported below have been made with this object in view.

All the experiments have been performed with Canton-S males and females at 25 ± 0.5° C. Individual males were mated to 8 females each day for 3 days, then to 5 females daily. After 24 hours with the male the females were isolated in separate vials which were subsequently checked for larvae and recorded as fertilized or unfertilized. The virgin females used were 2–3 days old. The results obtained are thus valid only for Canton-S, and some strain-to-strain variation is to be expected.

To test whether there was any accumulation of sperm in males stored without females, two series of matings were made in one of which 10 males were mated 0–4 hours after emergence while another 10 males were stored without females for 48 hours and then mated to new virgin females every 24 hours. The results presented in table 1 seem to establish conclusively that sperm are stored when males are kept without females, at least over a period of several days after emergence.

The next question was whether one female was sufficient to utilize mature sperm as they became available or whether several virgin females were necessary to achieve maximum sperm utilization. The latter alternative

TABLE 1

MEAN NUMBER OF FEMALES FERTILIZED PER DAY PER MALE WHEN MALES ARE MATED IMMEDIATELY AFTER EMERGENCE OR STORED FOR 48 HOURS

| Age of ♂ when first mated | Mating day 1 | 2 | 3 | 4 | 5 | 6 | Total |
|---|---|---|---|---|---|---|---|
| 0–4 hrs. | 2.6 | 3.6 | 4.6 | 3.7 | 3.0 | 2 | 19.5 |
| 48 hrs. | 8.5 | 6.5 | 4.0 | 1.0 | 2.3 | 1.5 | 23.8 |

proved correct in two further experiments. In the first of these, three groups of 10 males, 0–4 hours old, were mated as follows:

| | Mean no. ♀♀ fertilized 4th day |
|---|---|
| Group I: × 1 ♀ for 3 days | 6.8 |
| Group II: × 1 ♀ per day for 3 days | 6.5 |
| Group III: × 5 ♀♀ per day for 3 days | 4.0 |
| Groups I, II and III × 10 ♀♀ on 4th day | |

In the next experiment a mass mating of Canton-S females was made with Muller-5 males for 24 hours, after which the females were isolated individually in vials to test fertilization. After 2 days, 30 fertilized females were mated in groups of 10 to single Canton-S males for 24 hours, after which the females were again placed individually in vials. All the progeny of these females were checked and it was found that only one of the 30 females produced non-Bar daughters, *i.e.* progeny of the + males of the second mating. It seems that these non-virgin females are even less efficient for mating when they have once started laying eggs.

An experiment made primarily to test whether there was any difference in the mating pattern between untreated and irradiated males is illustrated in figure 1. The figure shows the mean number of females fertilized per day by three groups of males, one untreated, one irradiated with 500 r and one with 2500 r. Each group was started with 15 males but a good half of them had died before the end of the experiment. Some males from the untreated group and from the 500 r group were stored unmated for various periods to test sperm storage. These indicate that an isolated male, from the third day after emergence and thereafter, will always have enough sperm available to fertilize about 8 females during the first 24 hours of mating regardless of whether he is 3, 13, 17, or 21 days old. This means that copulation frequency is not so much dependent on the age of the male, within reasonable limits, as on the number of mature sperm available. Enough sperm for about 8 females thus seems to be the maximum of sperm storage, and beyond this amount it may be that sperm are ejaculated or resorbed without copulation. There is of course the alternative that maximum opportunity for copulation stimulates sperm production and that all sperm are stored in both cases. Further experiments are required to decide between these alternatives.

It is seen from figure 1 that the males treated with 2500 r showed very little activity on the 8th and 9th days after irradiation, only one female being fertilized on each of these days by 14 males mated to 5 females each.

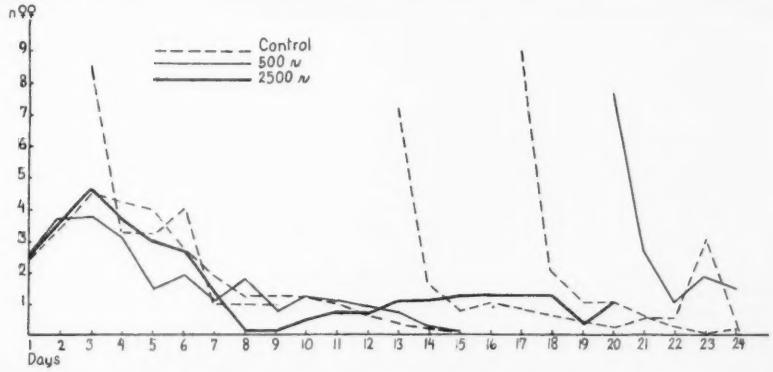


FIGURE 1. Mean number of females fertilized per day per male. Males mated on 1st day 0–4 hrs. old. Males mated for the first time on later days were removed from stock bottle at same time and were stored without females until mating day indicated on figure.

It is also noteworthy that subsequently these males fertilized more females than the other two groups, all of which had become sterile after the 15th day.

To check this drop on the 8th-9th days and also to control the *efficiency* of fertilization of these much-mated males, a similar experiment was carried out with untreated and irradiated males, with egg counts made from the isolated females. No eggs were collected during the mating period. Figure 2 shows the result.

Eggs were collected for 5 successive days from all the females, but only the first day counts have been used in the figure as fertilization was so "thin." The egg counts in the figure represent between 900–2500 eggs for each of the first 5 days, gradually falling off to around 100 for the last 5 days. Each series was started with 10 males. As an example of the enormous fertility of some males it might be mentioned that one male fertilized 799 eggs from 5 females during a single 24 hour period. The numbers of unhatched eggs—even in the untreated series—is quite high, between 10 and 25 per cent for most of the broods as compared to only 2–3 per cent unhatched in "normally mated" flies of the same Canton-S stock. Although this makes it difficult to calculate the percentage of dominant lethals induced in the irradiated flies, the constant decrease in number of eggs hatched, falling to zero on the 9th day, confirms the observation made in the previous experiment as regards mating frequency and affords a possible explanation of this as well. Cytological examination is necessary before it can be determined whether this drop in hatchability is due to dominant lethals or lack of functional sperm. In either case the sperm inseminated on these days have apparently been irradiated during the most sensitive period, and the intensive mating system has afforded a sharp distinction between broods from various stages. The sudden upward turn after the 9th day also indicates that this mating system affords sharper distinctions than

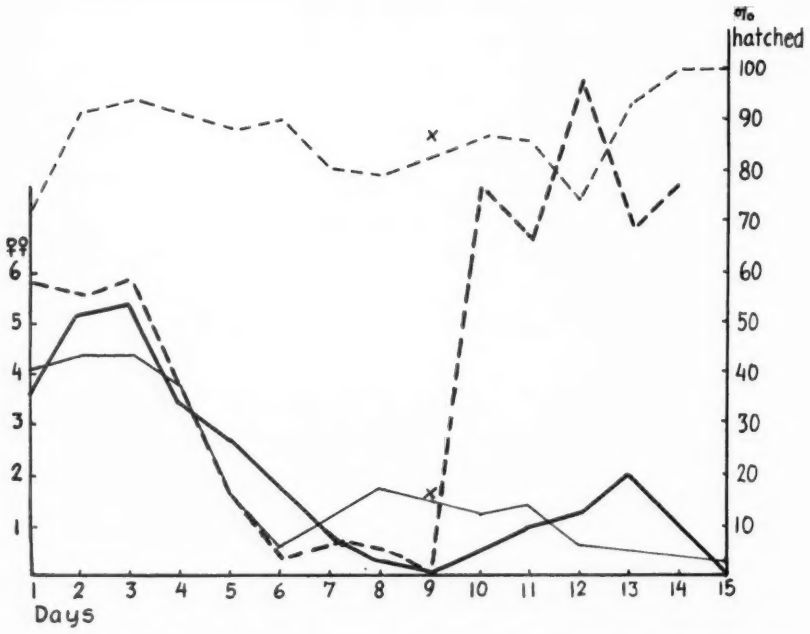


FIGURE 2. Mean number of females fertilized per day per male and % eggs hatched. (×) no control observations on 9th day due to accident.

♀♀ fertilized 2500 r
♀♀ fertilized control
% eggs hatched 2500 r
% eggs hatched control

those used previously. Dominant lethals might better be scored under this mating system by recording the number of flies hatched as an inverse measure of dominant lethality (King 1952).

The differing brood patterns for dominant lethals obtained by Fahmy and Fahmy (1954) with increasing doses of a chemical mutagen may possibly be an expression of the same phenomenon as that observed here. The toxicity of the higher doses may so reduce the number of sperm available that there is more complete sperm utilization at these higher doses, allowing an earlier and steeper fall to appear in the curves because there is less admixture with less sensitive stages not completely utilized in the broods at the lower doses.

For scoring visible or recessive lethals induced by radiation or by chemical mutagens, mating single males with at least 8 females on each of the first three days and thereafter with at least 5 females each day, it should be possible to separate the various stages of spermatogenesis, and this scheme may serve as a tool in identifying these stages more accurately.

SUMMARY

It is found that Drosophila males will fertilize as many as 10 virgin females per day and that, in order to achieve maximum utilization of sperm, it is necessary to mate males with large numbers of virgin females daily. It is demonstrated that mature sperm are retained in the testis at least a few days. When newly emerged males are irradiated with 2500 r and mated according to the above system, there is a decided decrease in mating activity and an increase in dominant lethals on the 6th to 9th day after irradiation.

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